Early impact of HPV vaccination at the population level: HPV genotypic prevalence in U.S. women from pre- and post-vaccine periods, 2003-2010

Michael H. Marco

Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2016

ABSTRACT

Early impact of HPV vaccination at the population level:
HPV genotypic prevalence in U.S. women from pre- and post-vaccine periods, 2003-2010

Michael H. Marco

The broad aim of this dissertation is to understand the early impact of HPV vaccination in females at the population level. Three important public health questions are addressed: 1) Is HPV vaccination doing what is expected: decreasing the prevalence of vaccine-type HPV 6, 11, 16 and 18 which are etiologically linked to invasive cervical cancer and genital warts?; 2) Is there evidence of beneficial cross-protection (decreased prevalence) of any of the other non-vaccine-targeted high-risk (HR) HPV genotypes?; and 3) With the expected decline of HPV 16 and 18, are there deleterious virological consequences, such as type-replacement (increased prevalence) with a rise of non-vaccine-targeted HR HPV? In the first chapter, I assess and synthesize peer-reviewed literature published from 2007 to 2013 that documented early impact of HPV vaccination. Seventeen ecological studies were stratified into three tiers based on degree of vaccination impact (cervical abnormalities, genital warts, and HPV DNA) and incidence or prevalence in samples from a pre-HPV vaccination time period (pre-2007) with that of a post-vaccination time period (post-2007) were compared. In the second chapter, I investigate vaccine-type HPV prevalence changes between pre- and post-vaccine periods in over 8,000 females aged 14-59 years enrolled in the NHANES HPV Vaginal Swab Surveys from 2003-2010. In the third chapter, I test for evidence of non-vaccine-targeted HR HPV genotypic cross-protection and type-replacement in NHANES Survey females from the pre- and post-vaccine periods. Finally, I conclude the dissertation with a summary of the findings and a discussion of the public health implications and offer suggestions for future research.

Table of Contents

List of Tables and Figures (excluding Appendix)

Acknowledgments

I would like to thank the members of my dissertation committee who have been extremely generous with their time and advice. Special thanks go to my advisor and sponsor, Stephen Morse, who believed in me and this project from the outset, and for demonstrating tremendous patience and generosity on so many levels. I also thank Heidi Jones for her help with thinking through HPV research issues and for being a warm and caring taskmaster.

In addition to my committee, I would like to acknowledge the continuous support I received from many friends and colleagues at Columbia's MSPH, including Mansi Agarwal, Stephen Arpadi, Ronald Bayer, Robin Flam, Gerald Oppenheimer, Sharon Schwartz, and Chloe Teasdale. I especially owe a huge debt of gratitude to Liliane Zaretsky for her guidance, hand-holding and warmth throughout this entire process.

Finally, I must acknowledge the encouragement, love and support of my friends and family, including Constance Benson, Lia and Joyce Brody, Carol Brosgart, Eli and Simon Caplan, Angela Garcia, Roger Frantz, Umesh Lalloo, Michael Morrill, Julie Myers, Ellen and Ron Jacobs, and Robert "Chip" Schooley. Most importantly, I offer heartfelt thanks to my touchstone, Dana March, who has been there for me every step of the way.

Dedication

This dissertation is dedicated to the memory of Alan Berkman (1945-2009), an activist, physician, teacher, and friend.

1.0 Chapter 1:

Introduction

Human papillomavirus (HPV) is a small double-stranded DNA virus, which infect cutaneous and mucosal epithelial cells. HPV is by far the most common sexually transmitted disease in the U.S. with approximately 6.2 million individuals infected yearly.[1] Sexual activity—often within the first 6 months of sexual debut—is the most pronounced risk factor for incident HPV infection in a female's genital tract.[2,3] HPV infection is age-dependent[4] and detected most often in sexually-active females aged 20-24 years.[5]

Of the approximately 120 HPV types that have been identified, 40 types sort into 15 alpha species which infect the genital tract.[6] These 40 HPV types are divided into two groups: high-risk (HR), also referred to as oncogenic or carcinogenic, and low-risk (LR). HR HPV genotypes have been etiologically linked to invasive cervical cancer (ICC), as well as vaginal, vulvar, penile, anal and a subset of head and neck cancers.[7-9]

ICC is the fourth most common cancer in U.S. females, with approximately 530,000 cases diagnosed yearly.[10] It is, however, the number one cancer in sub-Saharan African and South-east Asian females because high quality screening and early and effective treatment are sparse to non-existent.[11] In 2012, the World Health Organization (WHO) estimated that ICC was responsible for approximately 270,000 deaths worldwide.[10]

The WHO's International Agency for Research (IARC) acknowledges that there are 20 HPV HR genotypes with varying levels of carcinogenicity: HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82.[12] The most commonly detected HPV types found in ICC tissue, in descending order of frequency, are HPV 16, 18, 45, 31, 33, 52, 58, and 35.[8] HPV 16 and 18 are overwhelmingly the HR types most strongly associated with approximately 70% of ICC.[13,14] HPV 18, however, is most commonly detected in

adenocarcinoma of the cervix.[14,15] Approximately 40% of women who repeatedly test HPV positive (referred to as "persistent") for type-16 will go on to develop high-grade (precancerous) cervical intraepithelial neoplasia (CIN).[16] Moreover, there is a twelvefold increase in the risk of developing high-grade CIN in females with persistent HPV 16 and/or 18 infection compared to other HR HPV genotypes.[17] Stage-3 CIN left untreated for many years can invade the base membrane of the epithelium and cause cervical cancer.[18,19]

The 20 HPV types classified as LR HPV include HPV 6, 11, 32, 40, 42, 43, 44, 54, 55, 61, 62, 64, 71, 72, 74, 81, 83, 84, 87, and 89.[20] LR HPV 6 and 11 are associated with 90% of penile, vaginal, and anal warts.[21] Genital warts (GWs) are often the immediate clinical manifestation of incident HPV infection.[22] While GWs are easily treatable in an out-patient setting, the psychological burden caused by GWs, as well as the annual cost to health care systems, should not be underestimated.[23,24]

HPV prophylactic vaccination against HPV 6, 11, 16, and 18 has existed in the U.S. since 2006. Routine vaccination is recommended for all individuals aged 11 or 12 years, and catch-up vaccination for females aged 13 through 26 years and males aged 13 through 21 years.[25-27] The three U.S. Food and Drug Administration (FDA)-approved, commercially available vaccines differ in the number of specific HPV genotypes they prevent; however, all target HPV 16 and 18. Pivotal studies of the three vaccines reported 90-98% vaccine efficacy on all endpoints (e.g., CIN 2/3 or GWs) and all documented an excellent safety profile.[28,29 30,31]

Evidence of HPV genotypic cross-protection against cervical lesions due to HPV non-vaccine HR types in the phase III studies of the first two FDA-approved vaccines has been reported.[32,33] HPV type-replacement—increased prevalence of non-HR vaccine-type HPV—due

to a new ecological niche created by a reduction in the prevalence of HPV genotypes 16 and/or 18 is an important subject of concern being discussed in the HPV vaccine community.[34,35] To date, however, no data exist to support this occurring.[36,37]

With a median age of 49 years for ICC in the U.S.,[38] it will be approximately 25 years until significant clinical benefit of HPV vaccination—namely, a reduction in ICC incidence—can be established. Monitoring temporal trends in the prevalence of HPV 16 and 18 as well as other HR HPV genotypes is the surest and easiest way of monitoring early vaccine impact at the population level.[39] Hence, the broad aim of this dissertation is to understand the early impact of HPV vaccination in females at the population level. Three important public health questions will be examined: 1) Is HPV vaccination doing what is expected: decreasing the prevalence on vaccine-type HPV 16 and 18; 2) Is there evidence of beneficial cross-protection (decreased prevalence) of any of the other 20-30% of HR HPV genotypes etiologically linked to ICC?; and 3) With the expected decline of HPV 16 and 18, are there deleterious virological consequences, such as type-replacement with a rise in prevalence of other HR HPV genotypes?

Chapter 2 provides a systematic review that summarizes and synthesizes the population-based, early impact of HPV vaccination after licensure in 2006. While many systematic reviews and meta-analyses on the cost effectiveness of HPV vaccination exist,[40-43] there are no published systematic reviews investigating the early impact of HPV vaccination from countries with widespread vaccine utilization. This systematic review will also include background and a non-systematic review of evidence for potential biological mechanisms through which HPV vaccination may decrease or increase prevalence of either vaccine or non-vaccine-type HR HPV genotypes.

My review includes ecological studies in diverse female patient populations from the U.S., Europe, and Australia and New Zealand that compare virologic changes in LR and HR HPV genotypic prevalence as well as differences in the incidence of HPV clinically-related outcomes (e.g., GWs or CIN 2/3) between the pre- and post-vaccine eras. Documented changes in vaccine-type HR HPV prevalence and incidence of GWs before and after the advent of the vaccine are distinctive indicators for observing initial vaccine effectiveness.[39]

If a majority of the studies analyzed point in a positive direction, suggesting that vaccination is likely reducing the prevalence of vaccine-type HR HPV infection and decreasing GWs or cervical abnormalities in populations where the vaccine has been available for approximately ~10 years, pediatricians can use this information in a more proactive way to explain to parents the benefits of HPV vaccination for their daughters. In turn, increased female vaccination resulting from parental education of the vaccine's cancer-preventing qualities will undoubtedly help normalize and escalate HPV vaccine uptake in males for preventing anal, penile, and head and neck cancers.

Journal articles are categorized into three tiers according to degree of outcome/vaccination impact (clinical vs. virological changes) and qualify for review if they have data comparing outcomes on well-defined patient populations specifically before and after 2006. Studies with GW data on males are included and analyzed. Because HPV vaccination in males is relatively new, and widespread use is still uncommon, a decrease in heterosexual male GW incidence in countries where female vaccination coverage is high could suggest the effect of herd immunity.[44] Finally, the review adheres to practices as stipulated in the Preferred Reporting Items System for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[45]

In Chapter 3, I begin my ecological assessment of the relationship between HPV vaccination and the expected outcome of decreased prevalence of vaccine-type HR HPV in the pre- and post-vaccine eras (2003-2006 vs. 2007-2010). Unlike other analyses which specially focus on HPV prevalence in adolescents or include only a smattering of older females,[36,37,46] I explicitly aim to cast a wider net to look at a broader age range of females (age 14-59 years) and also categorize them by specific age groups. My analyses of temporal trends of HPV genotypic prevalence will specifically control for prior vaccination, race, education level, sexual activity, and marital status.

Chapter 3 also includes a geographical ecological assessment to determine if the prevalence of vaccine-type HR HPV now differs in the post-vaccine era between the top-10 U.S. states with the highest reported coverage of HPV vaccination in females compared with the bottom-10 states with the lowest reported coverage of HPV vaccination. I hypothesize that the top-10 states with the highest vaccine coverage will show a larger decrease in HR vaccine-type HPV prevalence in the post-vaccine period than the bottom-10 states with the lowest vaccine coverage. This would be another early indication that the vaccine is doing what it should.

Chapter 4 employs the same temporal trend methodology of examining HPV genotypic prevalence for evidence of non-vaccine-targeted HR HPV cross-protection and type-replacement in females at the population level. An analysis will be conducted to assess the relationship between vaccination and the beneficial outcome of cross-protection (decreased prevalence) of non-vaccine-targeted HR HPV genotypes from the alpha-7 and alpha-9 species. HPV 16 exists in the alpha-9 species that includes five HR HPV genotypes (HPV 31, 33, 35, 52, and 58), and HPV 18 is from the alpha-7 species which has four HR HPV genotypes (39, 45, 59, and 68).[47] Five in particular, HPV 31, 33, 45, 52, and 58, are etiological linked to approximately 20% of

ICCs.[13] Varying degrees of decreased prevalence of these five genotypes —signifying cross-protection—was documented in the phase III pivotal studies of the bivalent and quadrivalent HPV vaccines.[32,33]

In addition to my examination of HR HPV genotypic cross protection, I will test for type-replacement—increased prevalence of non-vaccine-targeted HR HPV from the alpha-7 and 9 species. It might be too early to document viral type-replacement while still in the first decade of wide-spread HPV vaccination. Nevertheless, examining this effect at the population level is prudent as it would provide public health officials with a robust sense of urgency for continuing to recommend routine cervical cancer screening in vaccinated females.

Unlike a previously published ecological study examining HR HPV cross-protection and type-replacement in females aged 14-19 years, my analyses will include those up to age 59 years. Lastly, all documentation of HPV prevalence will be adjusted for well known risk factors, including education, sexual activity, and marital status.

Chapter 5 includes a summary and discussion of the data analyses from my empirical papers and systematic review. It also explains the public health implications and offers recommendations for future research. The analyses planned for my dissertation add to an existing array of published mathematical modeling research[48-53] and a growing body of ecologic studies investigating the early impact of HPV vaccination.[36,37,46,54-57] Noteworthy differences in my research are an investigation of vaccine impact on a much broader age range of females and examining, for the first time, possible geographic differences in HR vaccine-type HPV prevalence based on rates of vaccine coverage. A significant strength of this research is the utilization of eight years of virological, behavioral, and demographic data from a well-

characterized population-based survey containing over 8,000 U.S. females. No other national survey from the U.S. or internationally with such rigorous sampling methods and excellent laboratory quality control comparing past and present HPV DNA prevalence exists.

This dissertation is an investigation of temporal trends of HR HPV genotypic prevalence before and after the advent of wide-spread HPV vaccination in females at the population level. The systematic literature review offers an in-depth look at the vast assortment of international ecological studies documenting differences in the incidence and prevalence of HPV virological and early-stage clinical outcomes between the pre- and post-vaccine eras. My empirical papers examine 1) the expected decrease in HPV vaccine-type prevalence; 2) the beneficial occurrence of non-vaccine-targeted HR HPV cross-protection; and 3) the deleterious effect of non-vaccine-targeted HR HPV type replacement. While it is too early to determine if HPV vaccination in females (and now in males) has had an impact in preventing cervical and other less common HPV-associated cancers, monitoring of changes in HPV prevalence at the population level is an integral part of the spectrum of initial outcomes of HPV vaccination which has obvious and important public health consequences in this HPV post-vaccination era.

References

1. Weinstock H, Berman S, Cates Jr W. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Repro H 2004;36:6-10.

2. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-8.

3. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsky LA. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218-26.

4. Franceschi S, Herrero R, Clifford GM, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer 2006;119:2677-84.

5. Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis 2009;200:1059-67.

6. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: human papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007:1-636.

7. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.

8. Muñoz N, Bosch FX, De Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.

9. Schiffman M, Castle PE, Jeronimo J, et al. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.

10. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. (Accessed November, 2013, at http://globocan.iarc.fr.)

11. Franceschi S, Denny L, Irwin KL, et al. Eurogin 2010 roadmap on cervical cancer prevention. Int J Cancer 2011;128:2765-74.

12. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.

13. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: A meta-analysis. Br J Cancer 2003;89:101-5.

14. de Sanjose S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.

15. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. Br J Cancer 2003;88:63-9.

16. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76-84.

17. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001;286:3106-14.

18. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol 1969;105:386-93.

19. Hawes SE, Kiviat NB. Screening for cervical cancer. In: Holmes KK, Sparling FP, Stamm WE, et al., eds. Sexually Transmitted Diseases. New York: McGraw-Hill Professional; 2007:1075-104.

20. Jacobs MV, De Roda Husman AM, Van den Brule AJC, Snijders PJF, Meijer CJLM, Walboomers JMM. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J Clin Microbiol 1995;33:901-5.

21. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol 1995;33:2058-63.

22. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191:731-8.

23. Pirotta M, Ung L, Stein A, et al. The psychosocial burden of human papillomavirus related disease and screening interventions. Sex Transm Infect 2009;85:508-13.

24. Hu D, Goldie S. The economic burden of noncervical human papillomavirus disease in the United States. Am J Obstet Gynecol 2008;198:500.e1-.e7.

25. Markowitz LE, Dunne EF, Saraiya M, et al. Human papillomavirus vaccination recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2014;63:1-30.

26. Petrosky E, Bocchini JA, Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: Updated hpv vaccination recommendations of the advisory committee on immunization practices. MMWR 2015;64:300-4.

27. CDC. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the advisory committee on immunization practices (ACIP). MMWR 2010;59:630-2.

28. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.

29. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374:301-14.

30. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med 2011;364:401-11.

31. Joura EA, Giuliano AR, Iversen O-E, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med 2015;372:711-23.

32. Brown DR, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; Types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. J Infect Dis 2009;199:926-35.

33. Wheeler CM, Castellsagué X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012;13:100-10.

34. Franco EL, Cuzick J. Cervical cancer screening following prophylactic human papillomavirus vaccination. Vaccine 2008;26 Suppl 1:A16-23.

35. Kahn JA. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med 2009;361:271-8.

36. Tabrizi SN, Brotherton JM, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645-51.

37. Kahn JA, Brown DR, Ding L, et al. Vaccine-type human papillomavirus and evidence of herd protection after vaccine introduction. Pediatrics 2012;130: e249-e256.

38. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2008. 2011. National Cancer Institute. Bethesda, MD, 2011. (Accessed June 1, 2014, at http://seer.cancer.gov/csr/1975_2008/.)

39. Hariri S, Markowitz L. Monitoring HPV vaccine impact: Early results and ongoing challenges. J Infect Dis 2012;206:1633-5.

40. Armstrong EP. Prophylaxis of cervical cancer and related cervical disease: A review of the cost-effectiveness of vaccination against oncogenic HPV types. J Manage Care Pharm 2010;16:217-30.

41. Fesenfeld M, Hutubessy R, Jit M. Cost-effectiveness of human papillomavirus vaccination in low and middle income countries: A systematic review. Vaccine 2013;31:3786-804.

42. Goldie SJ, Kohli M, Grima D, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. J Natl Cancer Inst 2004;96:604-15.

43. Techakehakij W, Feldman RD. Cost-effectiveness of HPV vaccination compared with Pap smear screening on a national scale: a literature review. Vaccine 2008;26:6258-65.

44. Brisson M, van de Velde N, Franco EL, Drolet M, Boily M-C. Incremental impact of adding boys to current human papillomavirus vaccination programs: role of herd immunity. J Infect Dis 2011;204:372-6.

45. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.

46. Markowitz LE, Hariri S, Lin C, et al. Reduction in human papillomavirus (HPV) prevalence among young women following hpv vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013;208:385-393.

47. De Villiers EM, Fauquet C, Broker TR, Bernard HU, Zur Hausen H. Classification of papillomaviruses. Virology 2004;324:17-27.

48. Choi YH, Jit M, Gay N, Cox A, Garnett GP, Edmunds WJ. Transmission dynamic modelling of the impact of human papillomavirus vaccination in the United Kingdom. Vaccine 2010;28:4091-102.

49. Baussano I, Garnett G, Segnan N, Ronco G, Vineis P. Modelling patterns of clearance of HPV-16 infection and vaccination efficacy. Vaccine 2011;29:1270-7.

50. Garnett GP, Kim JJ, French K, Goldie SJ. Chapter 21: Modelling the impact of HPV vaccines on cervical cancer and screening programmes. Vaccine 2006;24:S178-S86.

51. Marra F, Cloutier K, Oteng B, Marra C, Ogilvie G. Effectiveness and cost effectiveness of human papillomavirus vaccine: a systematic review. Pharmacoeconomics 2009;27:127-47.

52. Vanska S, Auranen K, Leino T, et al. Impact of vaccination on 14 high-risk HPV type infections: a mathematical modelling approach. PloS One 2013;8:e72088.

53. Regan DG, Philp DJ, Hocking JS, Law MG. Modelling the population-level impact of vaccination on the transmission of human papillomavirus type 16 in Australia. Sex Health 2007;4:147-63.

54. Brotherton JM, Fridman M, May CL, Chappell G, Saville AM, Gertig DM. Early effect of the HPV vaccination programme on cervical abnormalities in Victoria, Australia: an ecological study. Lancet 2011;377:2085-92.

55. Fairley CK, Hocking JS, Gurrin LC, Chen MY, Donovan B, Bradshaw CS. Rapid decline in presentations of genital warts after the implementation of a national quadrivalent human papillomavirus vaccination programme for young women. Sex Transm Infect 2009;85:499-502.

56. Leval A, Herweijer E, Ploner A, et al. Quadrivalent human papillomavirus vaccine effectiveness: a Swedish national cohort study. J Natl Cancer Inst 2013;105:469-74.

57. Oliphant J, Perkins N. Impact of the human papillomavirus (HPV) vaccine on genital wart diagnoses at Auckland Sexual Health Services. N Z Med J 2011;124:51-8.

2.0 Chapter 2:

Early impact of HPV vaccination at the population level: systematic review comparing HPV-associated virologic and clinical manifestations between pre- and post-vaccine periods

ABSTRACT

Background

Human papillomavirus (HPV) is a necessary cause of invasive cervical cancer (ICC). Vaccination against ICC began in 2006 with the commercial availability of the HPV quadrivalent vaccine, Gardasil. Gardasil is also targeted to prevent genital warts (GW). It will be approximately 25 years until significant clinical benefit of HPV vaccination—namely, a reduction in ICC incidence—can be established. However, scattered reports of decreased GW incidence in post-vaccine era populations have been published since 2009.

Methods

A systematic literature search of English language peer-reviewed journal articles published from 2007 to 2013 was conducted employing MEDLINE®, PubMed® and Scopus® to document the early impact of HPV vaccination. Studies were stratified into three tiers based on degree of vaccination impact (clinical vs. virological changes). Studies comparing incidence or prevalence in a pre-HPV vaccination time period (pre-2007) with that of a post-vaccination time period (post-2007) were included along with studies which included physician-verified vaccinated versus unvaccinated populations. While the primary goal of this review was to determine incidence or prevalence change in females (the overwhelming majority of vaccine recipients), studies which also included data on males were used.

Results

Of the 17 studies included in the final review, two detailed changes in cervical abnormalities, such as cervical intraepithelial neoplasia (tier 1); 11 described change in GWs

incidence or prevalence (tier 2); and four described change of HPV vaccine-type genotypic prevalence, i.e., HPV 6, 11, 16, or 18 (tier 3). Almost all of the studies were ecological in nature, with a few being cohorts (multi-clinic data analyses), and one using a cross-sectional design. Ten of 17 studies included patient vaccination status, and most mentioned that Gardasil was being used >80% in their patient population. Ten of the 11 HPV genital wart incidence studies included males with only three (all Australian) categorizing them as men who have sex with women (MSW) or men who have sex with men (MSM). All 17 studies from the three tiers documented varying amounts of decreased incidence or prevalence in their post-vaccine era patients, namely those in youngest categorized age groups ($\leq$21 years). Five documented decreased GWs in males. Interestingly, the three studies categorizing males by sexual orientation noted a decreased incidence in MSW but not in MSM.

Conclusion

Similar to the positive trend observed in published results of HPV vaccine mathematical modeling studies, all 17 studies analyzed in this review documented decrease in either HPV vaccine-like genotype prevalence, genital wart incidence, or cervical intraepithelial neoplasia at the population level. At a first glance ~10 years after the advent and widespread use of HPV vaccination, it is encouraging to observe decreased trends in HPV virologic and clinical abnormalities.

2.1. Introduction

Low-risk and High-risk HPV Associate with Genital Warts and Cervical Cancer

Of the estimated 120 human papillomavirus (HPV) genotypes identified, 40 types are known to infect the genital tract.[1] HPV is by far the most common sexually transmitted disease in the U.S. with approximately 6.2 million individuals infected yearly.[2] The 40 HPV types that infect the genital tract are categorized into two groups: high-risk (HR), also referred to as oncogenic or carcinogenic, and low-risk (LR). Up to 20 HR types[1] have been etiologically linked to invasive cervical cancer (ICC), vaginal, vulvar, penile, anal, and a subset of head and neck cancers.[3-10]

In the U.S., ICC is the fourth most common cancer in females, with approximately 530,000 cases diagnosed yearly.[11] It is, however, the number one cancer in sub-Saharan African and Southeast Asian females because high quality screening and early and effective treatment are sparse to non-existent.[12] In 2012, the WHO estimated that ICC was responsible for a approximately 270,000 deaths worldwide.[13]

Two HR types, HPV 16 and 18, are considered responsible for approximately 70% of ICCs worldwide.[14] HPV 16 is found in tissue in over 50-55% of ICCs whereas type-18 is most strongly associated with adenocarcinoma of the cervix.[14,15] The propensity for HPV 16 infection to lead to high-grade (precancerous) cervical intraepithelial neoplasia (CIN) compared to all other HR HPV types—individually or combined—has repeatedly been documented in large, well-designed international longitudinal screening studies.[16-18] In fact, approximately

[1] The 20 HPV genotypes considered to be HR: HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82.

40% of females who are HPV 16 persistent (repeatedly testing positive) will develop high-grade CIN within five years.[19]

The remaining 20 non-oncogenic HPV types[2] which infect genital tissue are considered to be LR.[20] LR HPV types 6 and 11 are associated with 90% of penile, vaginal, and anal warts.[21] In the 1999-2004 National Health and Nutrition Examination Survey (NHANES), 7.2% of females and 4% of males documented ever being diagnosed with genital warts (GW).[22] Another large population-based European study documented a higher rate: 10.6% of approximately 70,000 females sampled (aged 18 to 45 years) were reported to have a previous diagnosis of GW.[23] GWs are easily treatable in an out-patient setting. However, the psychological burden should not be underestimated, nor should the annual cost to health care systems.[24-27]

HPV Vaccination

There are currently three U.S. Food and Drug Administration (FDA)-approved, commercially available HPV prophylactic vaccines, Merck & Co.'s quadrivalent Gardasil and nonavalent Gardasil-9, and GlaxoSmithKline's Cervarix. Gardasil, Cervarix, and Gardasil-9 were licensed in 2006, 2009, and 2014, respectively. As per recommendations from the Advisory Committee on Immunization Practices (ACIP), HPV vaccination can be administered to girls 11 or 12 years of age with catch-up through age 26.[28,29] In late-2009, Merck's Gardasil received an additional indication from the FDA for Gardasil to be administered to males aged 9-26 years.[30]

[2] The 20 HPV genotypes considered to be LR: HPV 6, 11, 32, 40, 42, 43, 44, 54, 55, 61, 62, 64, 71, 72, 74, 81, 83, 84, 87, and 89.

Because Gardasil is a quadrivalent vaccine developed to protect against HPV types 16 and 18 as well as types 6 and 11, it is indicated for the prevention of cervical cancer, precancerous cervical lesions, vulvar, vaginal, and anal cancers, and genital warts in females. Cervarix, however, is a bivalent vaccine targeted to protect against HPV types 16 and 18 and is thus indicated solely for the prevention of cervical cancer and precancerous cervical lesions in females. In 2011, ACIP modified its recommendations of Gardasil for use in boys aged 11-12, with catch-up vaccination from 13 to 26 years, for the prevention of genital warts, anal cancer and precancerous lesions (anal intraepithelial neoplasia [AIN]).[31,32] The administration of both vaccines is identical; they are given in three doses at 1, 2, and 6 months.

Vaccine efficacy in Gardasil's pivotal phase III randomized placebo controlled study for prevention of CIN 2/3, adenocarcinoma in situ, or cervical cancer related to HPV 16 or 18 was 98% in the per-protocol susceptible population and 44% in an intention-to-treat population of all females who had undergone randomization (including those with and without previous HPV infection).[33] For Cervarix, the vaccine efficacy for a primary endpoint of HPV type 16 or 18, CIN 2/3, or adenocarcinoma in situ (CIN2+) documented in the pivotal phase III randomized placebo controlled study was 98.1%.[34]

Clinical trials employing Gardasil have also documented excellent vaccine efficacy in preventing GWs. One study in females[35] documented 95% vaccine efficacy, while another in males[36] observed 90% efficacy for preventing GWs.

In December 2014, Gardasil-9 became the most recent HPV vaccine approved by the FDA for use in females and males. It is a nonavalent vaccine, effective against nine HPV genotypes—the four from the original Gardasil, plus another five HR genotype: HPV 31, 33, 45,

52, and 58.[37] The pivotal study compared the original Gardasil to Gardasil-9 in 14,000 females aged 16 to 26 years that were followed up to 54 months. Noninferiority was achieved for HPV 6, 11, 16, and 18, and efficacy against persistent HPV infection and/or CIN 2+ caused by HPV 31, 33, 45, 52, and 58 was ~96%.[38]

HPV vaccination and evidence of cross-protection

Cross-protection (decreased prevalence) of five HR non-vaccine HPV types in the alpha-9 species (i.e., HPV 31, 33, 35, 52, and 58) was observed in the phase III pivotal studies of both HPV vaccines.[29,34,39-41] Because Cervarix is formulated with an adjuvant, cross-protection on non-vaccine HR types was expected. In analyzing data submitted by GlaxoSmithKline from its new drug application (NDA) for Cervarix, the FDA noted that in PATRICIA, the pivotal study, vaccine efficacy against CIN2+ due to any of 12 non-vaccine oncogenic types (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was 37.4%. Moreover, efficacy against HPV 31-related CIN2+ was deemed to be 89.4%.[29]

Documentation of non-vaccine-targeted HR HPV cross-protection from the receipt of Gardasil in its two phase III pivotal trials (FUTURE I and FUTURE II) was recently published.[39] In the pre-specified analysis, HPV genotypic cross-protection was also detected when combining all five HPV non-vaccine HR genotypes of the alpha 9 species (vaccine efficacy = 35.4%). Although the study was not powered to detect differences in individual genotypes, a statistically significant reduction in HPV 31 was observed.

HPV vaccination and the prospect of future HPV-type replacement

The concern that a new ecological niche could be created by a reduction in the prevalence of HPV genotypes targeted by the vaccines (i.e., HPV 16 and 18) is not without historic precedent. In the late 1990s and early 2000s, there was documentation of significant increases in the prevalence of non-vaccine serotypes that occurred after the wide-spread introduction of a heptavalent conjugate pneumococcal vaccine and a Bordetella pertussis vaccine.[42-46]

While it is an important subject of concern being discussed by the HPV vaccine research community,[47,48] there is no evidence of HPV type replacement in any of the pre-licensure HPV vaccine clinical trials to date. The recent approval of Gardasil-9 with its nonavalent structure has quelled concerns over type-replacement, however, it is important to remember over 200 million doses of the original Gardasil have been administered—mostly to females—since 2006.[49]

HPV Vaccine Coverage in the U.S., Europe and Australia

U.S.

From 2006-2011, U.S. HPV vaccine coverage steadily increased to a 53% completion rate of $\geq$1 doses in females aged 13-17.[50] However, the rate of coverage flatlined to 53.8% in 2011, and, in fact, a downward trend in coverage of $\geq$3 doses was noted between 2011 and 2012 (34.8% vs. 33.4%).[51] This setback to increase HPV vaccine uptake in females in the U.S. was met with great consternation from CDC leadership.[52] For boys, the CDC reported that 8.3% received $\geq$1 vaccine dose in 2011. However, the data on U.S. male vaccine coverage are incomplete as only 38% of states reported male HPV vaccination coverage.[50]

Europe

Published European HPV vaccine coverage data in females primarily exist from Scandinavian and Northern countries. In Denmark, for example, where free HPV vaccination programs with Gardasil started in 2008-2009 for 12-year-old females and later those up to 15 years, it is estimated that up to 85% of this population has received all three doses.[53] In Germany, where federally-funded HPV vaccine programs exist for females aged 12-17 years, vaccination appears to take place much closer to the end of the recommended age where coverage for females aged 16-18 years is ~40%.[54,55]

Australia

Since 2007, Australia has funded a free national HPV vaccine initiative (employing Gardasil) for females aged 12-13 years.[56] Later, two "catch-up" HPV vaccine initiatives took place between 2007–2009 for females aged 13-26 years. Vaccine coverage from the on-going free program is reportedly high, with $\geq$80% receiving one dose and $\geq$70% completing all three doses.[57] While data is sparse for vaccine coverage from the 2-year catch-up program, one study documented that approximately 58% of females aged 15-26 years received at least one dose.[58] And, similar to the U.S., vaccine coverage data on males are incomplete.

The Need for Early Impact of HPV Vaccination at the Population Level

It will be approximately 25 years until we can determine if HPV vaccination in females (and now in males) has had an impact in preventing ICC.[59] Hence, the monitoring of early vaccine HPV impact in the U.S., Europe, and Australia—namely, the reduction in cervical

abnormalities, genital warts or HPV genotypes 16 and 18 at the population level—is an integral part of understanding the potential impact of HPV vaccination in the interim. Thus, a systematic review of the literature which details changes in incidence or prevalence of HPV genotypes and clinical manifestations in the pre-vaccine era to that of the post-vaccine era is warranted.

2.2 Methods

Structure and Guidelines

This systematic review summarizes and synthesizes the population-based impact of HPV vaccination after licensure in 2006. It includes studies that have examined changes in the prevalence of LR and HR HPV genotypes in females from the U.S., Europe, and Australia in the pre- and post-vaccination periods; and virologic changes in the incidence or prevalence of HPV clinically-related outcomes (e.g., genital warts or cervical intraepithelial neoplasia [CIN]).

The review was undertaken per standardized practices as stipulated in the Preferred Reporting Items System for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[60]

Information Sources and Search Criteria

Materials for this review were identified by searching three electronic databases: MEDLINE® (Ovid), PubMed® (National Library of Medicine [NLM]), and Scopus® (Elsevier). Rather than a detailed list of myriad search terms, a wide net was initially cast using two MeSH headings: "papillomavirus" and "vaccines" for any peer reviewed journal published after 1 January 2007. Keywords were included with spelling variations and acronyms (e.g., HPV).

Table 1.1 details the refinement for including articles in this review using MEDLINE®. After including "papillomavirus" and "vaccines," the various terms were used to limit articles to only: 1) English language; 2) humans; 3) females; 4) 2007-2013; 5) not editorials or letters. To ensure capture of articles recently published in 2013 or published ahead of print, PubMed® was employed using the identical MeSH® headings and keywords, but only for articles published between 2012-2103. Finally, the same search of MeSH® headings and keywords was employed using the Scopus® database.

Eligibility Criteria and Study Selection

All citations with abstracts identified in MEDLINE®, PubMed® and Scopus® databases were transferred to Endnote® 6.0 (Thomson Reuters). After isolating and deleting duplicates in Endnote, a review of titles was conducted to assess for eligibility. Journal articles for this review were deemed eligible if:

- Both a pre- and post-vaccine period population were compared for virologic or HPV-related clinical outcomes;
- Physician-verified vaccination status data was available when vaccinated and unvaccinated patients were compared;
- Historic controls came from the same patient population (e.g., same STD clinic);
- None of pre- or post-vaccine patients populations were from previously conducted HPV vaccine RCTs (pharmaceutically sponsored or otherwise);
- GWs were vaginal or anal;
- HPV DNA data was collected in genotypic studies.

In cases of ambiguous titles, abstracts were consulted. Upon selection from title review, abstracts were further analyzed for possibly relevant articles. If articles were considered potentially eligible, a full-text read was commenced.

Data from each article's study were extracted using Excel. Data were collected on: 1) location; 2) study design; 3) data source; 3) sample size; 4) demographics (sex and age); 5) patient population comparison/s; 6) measures used for calculating exposure, outcomes, and covariates; and 7) statistical analysis employed.

Categorization of Articles

Articles were categorized into three tiers according to degree of outcome/vaccination impact (clinical vs. virological changes). Tier 1 included those articles with the outcome cervical intraepithelial neoplasia; Tier 2 included those which described change in genital wart incidence; and Tier 3 was composed of studies detailing change of HPV vaccine-type genotypic prevalence (HPV 6, 11, 16, or 18).

2.3 Results

Selection of Journal Articles Included in Systematic Review

A total of 2,369 unduplicated journal articles were selected for title review. (Figure 1 describes the flow of publications selected for review.) From these 2,369 articles, 327 abstracts were assessed. Twenty-eight full manuscripts were read and analyzed from the 327 abstracts. Of these 28, 11 were excluded from final selection because: 1) subjects were HPV vaccine clinical trial participants (N=5); 2) no specified pre-vaccine era population was included (N=4); 3) only HPV serology data collected (N=1); and 4) anal neoplasia was the only outcome data (N=1). Thus, 17 studies were included in qualitative synthesis.

Categorization and characterization of articles

Table 2 summarizes all 17 studies of early HPV vaccine impact comparing pre- and post-vaccine periods. When categorized, two articles detailed changes in cervical abnormalities, such as CIN [61,62] (tier 1); 11 described change in genital wart incidence[27,54,63-71] (tier 2); and four described change of HPV vaccine-type genotypic prevalence[72-75] (tier 3). The date of publications ranged from 2009 to 2013 with the first published on 16 October 2009 by Fairley and colleagues[66] and the last on 1 October 2013 by Blomberg and colleagues.[65] There were seven studies conducted in the U.S., five in Australia, four in Europe (two in Denmark, and one each in Sweden and Germany), and one in New Zealand. Twelve of the 17 studies employed an ecological study design. Ten included males and females while seven enrolled only females. The number of participants enrolled in these studies—or where database information was used—ranged from 225[72] to over 6 million[54]. Participants' age varied greatly across studies: three enrolled and/or allowed all ages, while others had ages ranging from 10 to 79 years.

Tier 1: Cervical abnormalities

The two studies evaluating vaccine impact on cervical abnormalities employed different study designs. One was an ecological study utilizing a state-based cervical screening register in ~4 million Australian females in which no individual HPV vaccination data were available.[61] The U.S. study was government-sponsored and employed an "indirect cohort" design with monitoring system surveillance data on ~5,000 females (75% with vaccine data history).[62] While the Australian study had a set population from the pre- and post-vaccine period, the U.S. study only had data on females after 2008. This study used individual data on vaccination status

for the comparison of the outcome. The outcome for both was CIN (high-or low-grade), however, the U.S. study was more exacting in that the lesions had to be DNA-positive for HPV 16 or 18. Results from the Australian and U.S. studies were similar: a decrease in high-grade CIN was observed between pre- and post-vaccine periods and unvaccinated and vaccinated females, respectively. Of note, a significant decrease in CIN incidence was only documented in females <18 years old from the Australian study.

Tier 2: Genital warts (GW)

Of the 11 GW studies, all but one employed an ecological study design.[65] A Danish study used a retrospective cohort design with ~400,000 females from birth cohorts from 1989 to 1999. The Danish Civil Registration System was linked to the National Health Insurance Service Register and the Prescription Registry for exposure and outcome data. Birth cohorts from 1989 to 1999 were selected because they had >10% HPV vaccination coverage ($\geq$1 dose). And unlike the 10 ecological studies which observed temporal trends in GW incidence or prevalence in a pre-vaccine versus post-vaccine period, the Danish cohort retrospectively studied vaccinated and unvaccinated girls and followed them for the occurrence of incident GWs. GW data on vaccinated and unvaccinated girls was then compared using Cox proportional hazards models. A statistically significant temporal trend in the relative risk of GWs was observed from oldest to youngest cohort. In fact, no incident GWs were documented in the youngest birth cohort.

Ten of the ecological GW studies measured incident infection while one study calculated annual prevalence.[67] When both females and males were included, all studies stratified by sex.

All studies stratified by age, however, there was substantial heterogeneity. Some only stratified dichotomously by age (e.g., <20 vs. ≥20; <28 vs. ≥28)[66,70] while others used multiples of 5-year age groups for their population.[54,67] Regardless of how stratification was determined, it proved necessary as all GW studies noted the most marked decrease in females from the youngest age group. Two studies noted that the statistically significant GW decrease was no longer observed in females old than 20 years of age.[54,70] Three found that females up to ages 28-30 had lower incidence of GW in the post-vaccine period compared to the pre-vaccine period.[66,68,71] And, one study found increased GW in females and males over 25 years of age.[64]

Data sources from the ten ecological studies come from two separate entities, sexual health clinics or large state or national patient databases or registries. Four were from sexual health clinics: three in Australia[27,66,71] and one in New Zealand.[70] Of the other six: two were in California (one private[67] and one public[64] insurance database); one was from the U.S. Defense Medical Surveillance System database of U.S. service members[69]; and the remaining three were national patient registries from Europe (Denmark,[63] Germany,[54] and Sweden[68]). As expected, the number of patients in the databases and registries was substantial, from ~1.5 million[69] to >6 million.[54]

There was considerable standardization in outcome measures for how GW incidence or prevalence was obtained. The four sexual health clinics specified that the diagnosis of GWs must be the first diagnosis and only in new patients. The databases and registries used a composite of IDC-9 and ICD-10 codes (delineating genital viral warts) and a National Drug Code (NDC) for pharmacy-dispensed imiquimod or podofilox. All ten ecological studies offered temporal trend analyses for outcome in GWs by year as well as between set pre- and post-vaccine periods (e.g., before and after 2006 or 2007).

As expected with ecological studies, individual HPV vaccination data was not available. Only one Australian study of patients from a sexual health clinic included prior HPV vaccination data on their subjects.[27] The data, however, are only self-reported and no information was offered as to attempts at physician verification. Nevertheless, the authors declare that their self-reported data are similar to previously-published Australian vaccination coverage data.

Only one of the 11 GW studies did not include males in the sample.[65] Table 2 separately analyzes data on males from the ten GW studies. Four did not specify the proportion of females and males enrolled. Five of the ten studies including males documented a statistically significant decrease in GW incidence in their youngest age groups.[27,64,66,70,71] Interestingly, three studies (all Australian) categorized and independently analyzed data on males if they had sex with women (MSW) or sex with men (MSM).[27,66,71] In all three studies, it was only the MSW who were found to have a decrease in GW incidence.

HPV infection

Four studies documented change in prevalence of HR HPV infection between pre- and post-vaccine periods.[72-75] Three were conducted in the U.S., while the other was done in Australia.[75] Two were prospective cohort studies employing historic controls.[72,73] Both were conducted in the U.S. at university medical centers with females enrolled at either community health centers or STD clinics. They used a previously-enrolled, pre-vaccine period cohort for their comparison group. Neither had more than 800 patients. The Australian study was similar in the fact that it used a repeat cross-sectional design collecting HPV DNA samples on two cohorts (~600 females) from two time periods—2005-2007 (pre-vaccine) and 2010-2011 (post-

vaccine).[75] The largest study by far employed an ecological study design.[74] It was conducted by the CDC using its NHANES data from four 2-year cycles of HPV DNA vaginal swab samples (2003-2010). It comprised >8000 females aged 14-59 equally divided into pre- and post-vaccine periods who were analyzed by age groups (14-19; 20-24; 25-29; 30-39; 40-49; and 50-59 years old). While the methods are virtually identical across all four surveys, the one significant difference was that the 2003-2004 and 2005-2006 surveys over-sampled females aged 14-19 years (n=1,363) but did not for 2007-2008 and 2009-2010 (n=740). No explanation is offered as to why the over-sampling of this age group was discontinued.

The upper-limit age in the three non-ecological studies was much lower than in the NHANES study. One study[72] enrolled females only up to 17 years of age, while the other two allowed females up to age 24-26 years.[73,75]

One of the four studies simply compared all HPV types and HR vaccine-type (HPV 16 & 18)[72] while the other three attempted to compare prevalence data on all HPV types (using the Hoffmann‑La Roche Linear Array® Assay), LR VT-HPV (6 & 11), HR VT-HPV, and non-VT-HPV. This larger comparison was done in order to determine if there was evidence of cross protection (decreased HPV prevalence) as well as possible type-replacement (increased HPV prevalence) of HR HPV types.

There was heterogeneity in collection of HPV vaccination history data. The two smaller U.S. cohort studies had physician-verified data while the Australian and NHANES studies used self-reported vaccine history.

All four studies documented a statistically significant decrease in the primary endpoint of HR VT-HPV between their pre- and post-vaccine period patients. In the NHANES study, a

significant decrease in prevalence in HPV 16 and 18 between the pre- and post-vaccine periods was only documented in females aged 14-19 years (prevalence ratio: 0.50; 95% CI: 0.34-0.74 [$p<0.001$]).

No study observed a statistically significant decrease in non-vaccine targeted HR HPV between the two periods, thus ruling out a finding of cross-protection between HPV 16 and 18 and other genotypes that are part of the alpha 9 species. With regard to the NHANES data, the fact that the pre-vaccine group was over-sampled with 1,363 females while the post-vaccine group was not and contained only 740 females may have underpowered the ability to document cross-protection in the 14-19 year old females.

Of the three studies which also looked for possible type-replacement with increased prevalence of non-vaccine targeted HR HPV from the alpha 9 species, only one of the cohort studies[73] documented increased prevalence (60.7%–75.9%) in females who had been vaccinated compared to those who had not.

2.4. Discussion

Results from all 17 studies analyzed in this systematic review demonstrate that at the population level, HPV vaccination which began in 2006 has had an immediate impact in reducing cervical abnormalities, GWs, and the prevalence of HPV vaccine-type infections. These results mimic the positive virologic and clinical outcomes of HPV vaccination that have been widely documented in numerous mathematical modeling studies.[76-90]

Of substantial importance, and bringing to realization specific results of modeling studies,[87,89,90] five of the ten GW studies with male subjects documented decreased incidence or

prevalence.[27,64,66,70,71] HPV vaccination coverage for males in these studies (three from Australia, one from New Zealand, and the other from the U.S.) was never documented to be over 5%. One explanation for this finding is that vaccination of young females provided some form of herd immunity to young males. The most salient finding which lends credence to the herd immunity hypothesis comes from the three Australian GW studies[27,66,71] that categorized males based on their sexual preference for females or other males. All three studies noted a statistically significant decrease in GWs in MSWs but not in MSMs.

This finding was not replicated in studies outside of Australia, as only the Australian studies distinguished between MSWs and MSMs and utilized data from sexual health clinics. The only other study whose participants came from a sexual health clinic did not ask nor classify males based on their sexual preference.[70] And understandably, national health or insurance databases, which were used in the remaining seven GW studies, do not have such specified information on their patients.

The field of researchers from various parts of the world conducting these surveillance studies is still quite small. While 17 studies on the early impact of HPV vaccination at the population level were identified, only five of the papers had independent, non-overlapping masthead authors.[54,67-70] The two Danish studies had the same last author from the Danish Cancer Society Research Center.[63,65] Of the five Australian studies, there were only two separate groups of researchers. Four of the seven U.S. studies were conducted and/or sponsored by the CDC's Division of STD Prevention,[62,64,67,74] and a fifth was done by the Department of Defense (DoD) with editorial assistance from one of the CDC's leading HPV experts.[69] The two other U.S. studies, which were based on cohort studies implemented by university medical centers in Ohio[73] and Indiana,[72] included overlapping researchers.

The debate of using incidence versus prevalence for "newly detected" HPV in ecological and cross-sectional studies has been discussed in the literature for many years.[3,91,92] This is because without long-term follow-up from longitudinal studies of females at or before the time of sexual debut, it is impossible to distinguish whether newly detected HPV infection is, in fact, a new acquisition or merely a reactivation of latent infection.[93] HPV infection is not a constant; it is dependent on the interval between tests. In fact, no assay currently exists to identify past/cleared HPV infection, the duration of given infection, or if a particular HPV genotype is either new or a reinfection. Likewise, clearance of HPV DNA, at least to the point of non-detectability, in younger females, is common. One longitudinal study[94] documented that 60% of women with LR HPV cleared their infection within 10 months, while another study[95] found that females cleared their infection at 12% per month.

Measurement of GWs can be as unpredictable as HPV DNA. GWs are often the immediate clinical manifestation of incident HPV infection.[92] Many studies have documented that GWs are known to spontaneously clear in the absence of treatment, most likely because of acquired cellular immune responses.[96-98] Moreover, many individuals with genital warts—either raised (exophytic) or flat—are completely unaware of them.[99]

Measurement error is likely in studies utilizing databases that document particular ICD codes and/or prescriptions for imiquimod or podofilox. In fact, authors from one study analyzed admit that their administrative database used for detecting GWs before 2007 was "incomplete and unreliable."[64] Interestingly, they never mention if or how it was fixed.

There should also be a dose of skepticism in trusting the rate of GW diagnosis in the GW studies. It would be prudent to ask: Has uptake in cervical cancer screening significantly

changed in various countries in the post-vaccine era or between HPV vaccinated and non-vaccinated women? In one of the GW studies which documented an increase in GWs in older women in the post-vaccine era, the authors question if that finding is not simply due to increased health care-seeking behavior as a result of heightened awareness of HPV and its clinical manifestations.[64] With regard to clinicians who perform cervical cancer screenings in post-vaccine era, it is uncertain if knowledge of a patient's vaccine status leads them to more or less closely inspect for GWs during examination.

Another flaw apparent in many of the GW studies was not differentiating Gardasil from Cervarix and claiming that HPV vaccination (as single entity) was reasonable for the observed decrease in GWs. This is a small yet not insignificant issue when claiming the exposure, simply "HPV vaccination," was responsible for the outcome, a temporal change in GWs. Gardasil is the only vaccine which protects against HPV 6 and 11, the genotypes responsible for 90% of GWs. There is no biologic plausibility that Cervarix would alter GW prevalence. Nevertheless, because Gardasil was approved in all countries represented in this analysis two years before Cervarix (~2007), it does make the exposure-outcome claim relevant for females vaccinated before 2009. In fact, researchers of the ecological study from Germany point out that ~90% of females have been vaccinated with Gardasil as opposed to Cervarix.[71] Some researchers, however, contend that the current wide use of Cervarix will complicate future efforts to study the effect of HPV vaccination on GW prevalence.[67]

An issue worth exploring in these studies is whether sexual activity of young females has significantly decreased in the post-vaccine era, and, in turn, might be responsible for the decrease seen in all GWs studies. A few researchers considered this in the discussion of their study results. One from Sweden[68] noted surveys showing an increase in lifetime sex partners in both

females and males as well as increases in gonorrhea cases among both sexes. Another researcher from one of the Australian studies revealed that at the Melbourne Sexual Health Centre, there was an absence of any significant change in initial diagnoses of genital herpes between 2004 and 2008 as well as a consistency in the number of sexual partners reported for females aged less than 28 years.[66]

All four HPV DNA infection studies included a population from the pre-vaccine era and one from the post-vaccine era. Likewise, all attempted to delineate the number of females who were vaccinated versus not in the later time period. In the two U.S. HPV DNA infection studies,[73,74] the proportion of females receiving at least 1 vaccine dose was markedly different. In the Ohio cohort study, 59.2% received $\geq$1 dose compared to ~21% of similarly-aged females in the NHANES sample. Interestingly, the vaccine rate in both studies differs from the U.S. national average of 48.7% as reported in the CDC-sponsored 2010 NIS-Teen Study.[51] Neither study discusses the incongruity of vaccination coverage in their samples compared to the national average.

Three of the four HPV infection studies also compared prevalence between females reporting vaccination versus those not.[73-75] All three studies included data on the self-reported number of doses of their participants, and defined "vaccinated" as receiving at least 1 dose. Grammatically this is correct, yet internationally, all guidelines stipulate that HPV vaccination must consist of a series of 3 injections over 6 months. There are a few studies—those with serological endpoints of vaccine titers—which have suggested that one to two doses may be similarly as effective as all three doses.[100,101] However, classifying both one dose and three doses together as "exposed" has been met with some misgivings in the literature.[102]

Categorizing levels of vaccine exposure three ways, “not exposed,” “one or two doses,” or “all three doses,” would be more precise.

All HPV infection studies had enough power to demonstrate prevalence change in HPV infection when grouping vaccine-type genotypes 6, 11, 16, and 18. When attempting to document prevalence change in individual HPV types, some fell short. This was most pronounced in the NHANES study, which chose to compare individual genotypes only with their subset of 14-19 year old females.[74] Indeed, individual comparisons were hampered because NHANES chose not to oversample this age group between 2007-2010 (n=738) as it had done between 2003-2006 (n=1363). There was a relative standard error of $\geq$30% or less than 10 observations for vaccine-type 11 and 18 as well as 5 for the 6 genotypes from the alpha-9 species.

A significant strength of this systematic review is its expansive approach of combining three different characteristics of early HPV vaccine impact: a decrease in cervical abnormalities, GWs, and genotypic prevalence. Except for a recently published brief summary on early HPV vaccine impact,[103] this is the first systematic review on the subject which adhered to standardized practices as stipulated in the Preferred Reporting Items System for Systematic Reviews and Meta-Analyses (PRISMA).[60] And as stipulated, there was external quality control of the data collection for this review. As with all systematic reviews, it may be subject to publication bias in which early HPV impact studies with null or negative findings are possibly absent from the published literature.

Overall, this review found sufficient evidence to support an association of HPV vaccination in females with a decrease in HPV-related virologic and clincial manifestations at

the population levels. Because it will be approximately 25 years until effectiveness of HPV vaccination—as evidenced by a marked decrease in the rate of ICC—can be demonstrated, these findings are essential to monitor the early and intermediate effects of HPV vaccination. It should be reassuring that all 17 HPV studies from six countries analyzed in this sytematic review pointed in a positive direction; that vaccination is likely reducing the prevalance of HR HPV infections and that there has been an observed significant decrease in GWs and cervical abnormalities in populations where the vaccine has been availabe for ~7 years. With the recently published data on the vaccines's safety[51] as well as the results from these 17 studies, pediatricians should make a concerted effort and take a more active role in discussing with parents the many benefits of HPV vaccination for their children.

2.5 Tables and Figures

Table 2.1. Search criteria for MEDLINE® (Ovid)

1. exp Papillomavirus Vaccines/
2. hpv.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
3. human papilloma virus.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
4. 2 or 3
5. vaccin*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
6. 4 and 5
7. 1 or 6
8. limit 7 to (english language and female and humans and yr="2007 - 2013")
9. limit 8 to (editorial or letter)
10. 8 not 9

Figure 2.1. Diagram of the systematic review's search strategy and results

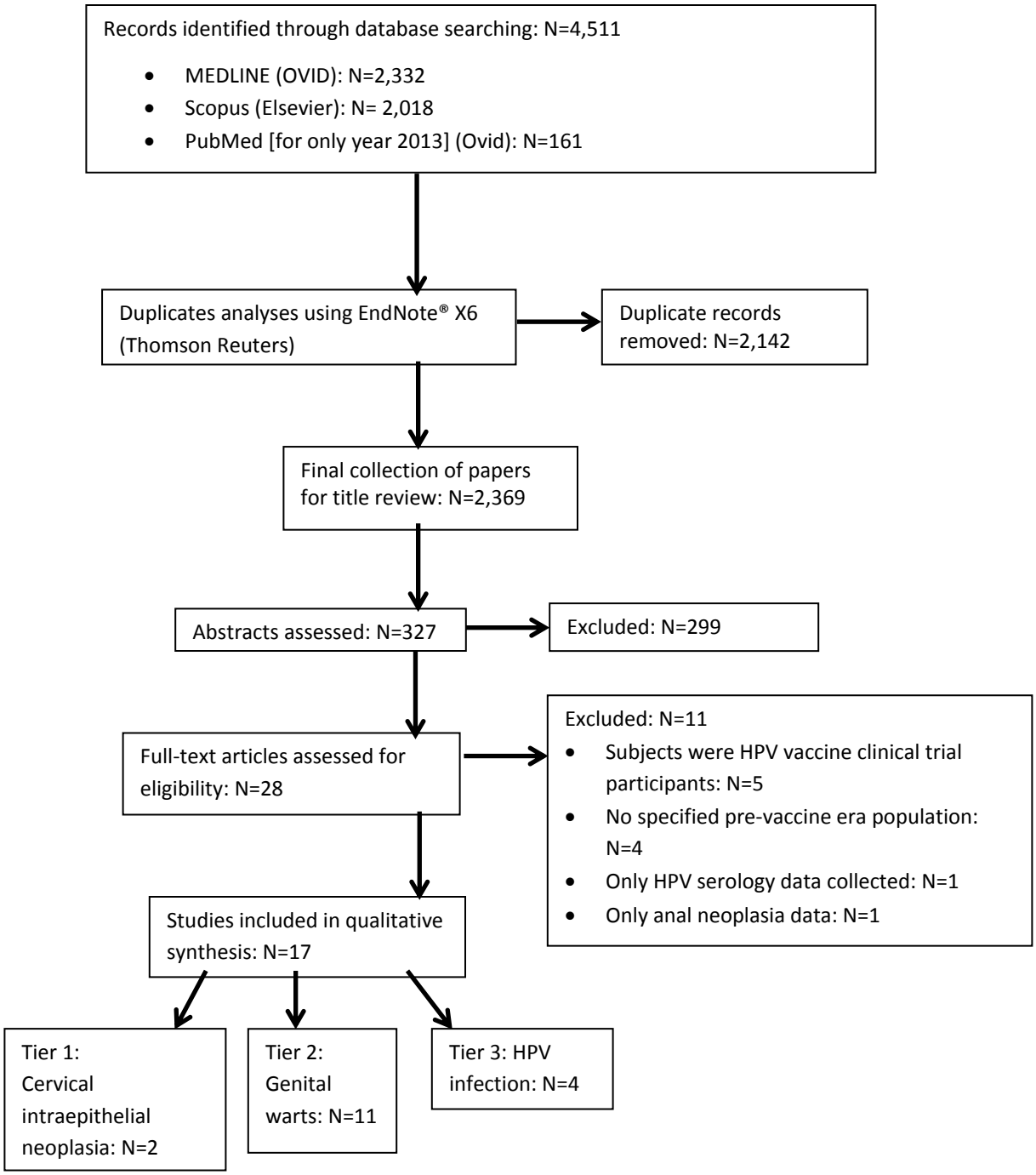


Table 2.2. Summary of studies of early HPV vaccine impact comparing pre- and post-vaccine periods

Tier	First author, year & ref#	Location	Study design	Data source and years of coverage	N =	Population(% female)	Age range	HPV vaccination data available	Comparison	Results[a]
Tier 1: Cervical abnormalities (N=2)	**Brotherton, 2011**[61]	Australia	Ecological	Victorian Cervical Cytology Registry 1/1/2003-12/31/2009	3,983,024	Females only	<17 - >31	No	Change in incidence of HGA & LGA detected by screening): 1/1/2003-4/31/2007 and 5/1/2007-12/31/2009	Decreased incidence of HGAs by 38% in <18 y/o. NS difference of HGAs in older females or in LGAs at any age
	Powell, 2012[62]	U.S.	Ecological	Surveillance data from the HPV-IMPACT monitoring system in CA, CT, NY, OR, & TN; 2008-2011	5,083	Females only	18–31	Yes: vaccination history data on 3,855 individuals (75.8%)	Time interval between vaccination and abnormal pap smear screening between 2008-2011	Decrease in HPV 16 and 18-positive CIN lesions in those with an abnormal pap smear receiving 1 vaccine dose ≥24 months prior compared to non-vaccinated

Tier 2: Genital Warts (N=11)	**Baaudrup, 2013**[63]	Denmark	Ecological	Danish National Patient Register (Data from 1/1995 – 7/2011)	All Danes (number not reported)	Males & females (67%)	12-≥36	No	Difference in the incident rate (IR) of genital GW before and after 10/2006	Decreased IR of GW 6/2008 to 6/2011 in females: 16 - 17 y/o 381.5 to 39.8 per 100,000 PYs; smaller yet significant decrease in those 18-29 y/o. NS difference in all males
	Bauer, 2013[64]	U.S.	Ecological	California Family Planning Access Care & Treatment (Family PACT [serves low-income adults]); database analysis of encounter claims 2007-2010	≥2,012,000	Males and females (87%)	All ages	No	Difference in GW incidence between 2007 - 2010 in patients with no record of previously GW diagnosis; stratified by year, sex, and age (< 21; 21-25; 26-30; >31 y/o)	Decreased GWs diagnosed 2007-10 in females: (<21 y/o [9% to 6%]; 21-25 y/o [1% to .9%]); and males: (<21 y/o [2.7% to 2.2%]; 21-25 y/o [5.1% to 4.5%]). GWs increased in older females and males 26-23 y/o.

Tier 2: Genital Warts Con't. (N=11)	**Blomberg, 2013**[65]	Denmark	Ecological	Population-based Civil Registration System of birth cohorts born 1989-1999	399,967	Females only	13.2-18.7	Yes; N=248,800 [62.2%] from 10/2006-5/2012; 14-90% received 1 vaccination; varied by cohort	Difference in GW incidence in vaccinated vs. unvaccinated females	HR by birth cohort for vaccinated vs. not: 1989-90 (.62); 1991-92 (.25); 1993-94 (.22); 1995-96 (.12); 1997-98 (no event [GW])
	Donovan, 2011[27]	Australia	Ecological	8 urban sexual health services spread throughout the country; (Data from 1/2004-12/2009)	112,083	Males and females (44%)	12-26	Yes (self-reported)	Difference in proportion of patients with first diagnosis of GWs in pre-vaccination (1/2004-6/2007) vs. post-vaccination period (7/2007 - 12/2009	GWs in females decreased from 11.7% to 4.8%. 12.3% -to 8.9% decrease in GWs observed in MSW. No change in MSM

Tier 2: Genital Warts Con't. (N=11)	**Fairley, 2009**[66]	Australia	Ecological	GW at Melbourne Sexual Health Center; 2004-2008	36,055	Males and females (~40%)	All ages	No	Difference in GW incidence proportion of new clients not previous diagnosed between pre-vaccination and post-vaccination periods: 2004-07 and . 2008); stratified by age (>28 or <28 y/o)	GW incidence in females <28 years decreased 12.7% to 6.6% between the pre- and post-vaccine eras. Decrease also observed in MSW (14.3% to 11.8%). No difference noted in females >28 years or MSM
	Flagg, 2013[67]	U.S.	Ecological	Truven Health Insurance Analytics MarketScan Commercial Claims and Encounters Database (2003-11)	≥64 million patient years of data	Males and females (51.4%)	10-39	No	Difference in annual GW prevalence and in pre- and post-vaccine periods; stratified by sex & 5-year age groups	GW prevalence per 1000/pys decreased in females 2006-10: (15-19 y/o: 2.9 to 1.8). Increase in males 15-39 y/o 2003-09, stable in 2010

Tier 2: Genital Warts Con't. (N=11)	**Leval, 2012**[68]	Sweden	Ecological	Swedish National Patient Register and Prescribed Drug Register; 2006-2010	Entire population with in age range (~4.2 million)	Males and females (% not reported)	10-44	No	Difference in annual GW incidence proportion and in pre- and post-vaccine periods; stratified by sex & age	GW incidence per 100,000/pys decreased in females 2006-2010: (15-19 y/o: 617 to 532; 20-24 y/o: 1,038 to 855). NS difference in older females or males.
	Mikolajczy, 2013[54]	Germany	Ecological	German pharma-epi research database (analysis of a large health insurance company: 2005-08)	≥6 million	Males and females (~45%)	10-79	No	Difference in IR of GW before and after 10/2006; stratified by 5yr age groups & sex	GW incidence per 100,000/pys decreased 316 to 242 in 15-19 y/o females 2007-2008. NS difference in older females or males

Tier 2: Genital Warts Con't. (N=11)	**Nsouli-Maktabi, 2013**[69]	U.S.	Ecological	The Defense Medical Surveillance System (database analysis of U.S. service members 2002-12)	1,440,362-1,544,029	Males and females (% not reported)	>17	No	Difference in the IR of GWs before and after 10/2006	GW IR per 100,000/pys decreased in females 2006-2010: (17-20 y/o: 3,576 to 2,143; 21-24 y/o: 2,700 to 2,027). NS difference in older females or males
	Oliphant, 2011[70]	New Zealand	Ecological	Auckland Sexual Health Service (database analysis of first-time patients: 2007-6/2010	40,793	Males and females (% not reported)	All ages	No	Difference in GW diagnosis for first-time patients between 1/2007-12/2008 dichotomous stratification by age >20 and <20 y/o	Decrease in GW diagnoses between time periods greater in females <20 y/o (13.7% - 5.1%) versus >20 y/o (7.5% - 5.9%); difference only in males >20 y/o: 11.5% - 6.9%)

Tier 2: Genital Warts Con't. (N=11)	**Read, 2011**[71]	Australia	Ecological	Melbourne Sexual Health Centre (data- base analysis of first-time patients diagnosed with GWs: 7/7/2004 – 6/30/2011)	52, 454	Males and females (% not reported)	All ages	No	Difference in GW diagnosis for first-time clinic patients between 1/7/2004 to 6/30/2011 ; cut- point 1/7/2007; stratification by age: <21; 21-29; and >30 y/o	Decrease in GWs diagnosed 2007-08 to 2010-11 in females <21 y/o (18.6-1.9%) and in MSW <21 y/o (22.9%-2.9%); NS difference in older females and MSM
Tier 3: HPV Infection (N=4)	**Cummings, 2012**[72]	U.S.	Ecological	Indiana University School of Medicine: Young Women's Project (YWP) 1995-2005, and post-vaccination prospective era cohort (PC), 2010.	225 (75 from the PC each matched with 2 historical controls (150) from the 387 in the original YWP. Both cohorts recruited from 3 urban STD clinics	Females only	14-17	Yes; on all 75 in PC (self-reported & physician verified): 8 (10.7%) not vaccinated; 8 (10.7%) had one vaccine dose, 10 (13.3%) had two, and 49 (65.3%) completed the series	Difference in prevalence of vaccine-type (VT) HPV DNA (6, 11, 16 or 18) from vaginal swabs in females from the YWP and PC	Prevalence of VT HPV decreased between the YWP and the PC: 24% to 5.3% ; decrease was also observed for only HPV 16 and 18: 16.7% to 5.3%

Tier 3: HPV Infection (N=4)	**Kahn, 2012**[73]	U.S.	Ecological	Ohio; females from commu-nity health clinics: pre-vaccine cohort (Pre-vax) recruited 10/2006 to 5/ 2007, post-vaccine cohort (post-vax) 12/2009 to 6/2010	777 (368 pre-vax cohort & 409 post-vax cohort)	Females only	13-26	Yes; documented though the statewide immuni-zation registry; data confirmed on 354 of 409 (87%) females; 242 (59.2%) received at ≥1 vaccine dose	Difference in cervico-vaginal HPV DNA prevalence between the pre-vax and post-vax cohorts	Prevalence of VT HPV decreased between pre-vax and post-vax cohorts: 31.7% to 13.4%; non-VT type HPV increased in females who had been vaccinated: 60.7%–75.9%
	Markowitz, 2013[74]	U.S.	Ecological	NHANES; data from 4 consecutive 2-year HPV DNA vaginal swab surveys (2003-2010)	8,403: 4,150 from the 2003-4 & 2005-6 (pre-vaccine period population) and 4,253 from the 2007-8 & 2009-10 (post-vaccine period population)	Females only	14-59	Yes; self-reported for the post-vaccine period population	Difference in cervico-vaginal HPV DNA prevalence between the pre-vax and post-vax periods	Prevalence of VT HPV decreased only in females 14-19 y/o between pre-vax and post-vax periods: 11.5% to 5.1%

Tier 3: HPV Infection (N=4)	**Tabrezi, 2012[75]**	Australia (3 major urban centers)	Repeat cross-sectional HPV DNA prevalence study (conducted in 2 periods: 2005-6 and 2010-11	Females attending family-planning clinics (FPCs) for routine pap smears	606 (202 from the pre-vaccine group and 404 from the post-vaccine group)	Females only	18-24	Yes; self-reported; 338 [85.6%] received ≥1 vaccine dose	Difference in cervico-vaginal HPV DNA prevalence between the pre- and post-vaccine groups	Prevalence of VT HPV decreased between the pre- and entire post-vaccine groups: 28.7% to 6.7% . Prevalence all was also less in vaccinated females compared to those not: 5.0% vs. 15.8%

-Abbreviations: ref: reference; LGA: Low-grade [cervical] abnormalities; HGA: High-grade [cervical] abnormalities; CIN: cervical intraepithelial neoplasia; GW: genital warts; IR: incidence rate; yr: year; y/o: year/s old; MSW: men who have sex with women; MSM: men who have sex with men; STD: sexually transmitted disease; vax: vaccine or vaccinated; VT: vaccine type (HPV 6, 11, 16, and 18); NS: non-statistically significant

- Unless specified, pre-vaccine period is defined as any time before 31 December 2006
- Unless specified, post-vaccine period is defined as any time after 1 January 2007
- Unless specified, all results are statistically significant at ≤0.05

Table 2.3. Data on males from the genital warts ecological incidence studies

First author, year and ref#	Country	Time Periods Measured	HPV Vax info on males?	Categorized MSW or MSM?	Outcome
Baaudrup, 2013[63]	Denmark	6/2006 – 7/2011	No	No	No significant change observed in any age group
Bauer, 2012[64]	U.S.	2007 - 2010	No	No	Prevalence decreased 19% in <21 y/o and 10% in 21-25 y/o. No change observed in older age groups
Donovan, 2011[27]	Australia	1/2004-12/2009	No	Yes	From 7/2007-12/2009, incidence decreased from 12.3% to 8.9% in MSW ≤26 y/o. No change was observed in MSM
Fairley, 2009[66]	Australia	1/2004-12/2008	No	Yes	In 2008, incidence decreased 5% in MSW. No change was observed in MSM
Flagg, 2013[67]	U.S.	1/2003 - 12/2010	No	No	Incidence increased for 15-39 y/o from 2003-2009, but then stable in 2010
Leval, 2012[68]	Sweden	2006-2010	No	No	No change was observed in any age group
Mikolajczyk, 2013[54]	Germany	2005-2008	No	No	No change was observed in any age group
Nsouli-Maktabi, 2013[69]	US	2000-2012	No	No	No change was observed from 2000-2009; incidence increased from 2010-2012 in all age groups
Oliphant, 2011[70]	New Zeeland	1/2007 – 6/2010	No	No	From 2007 - 2010, incidence decreased from 11.5% to 6.9% in <20 y/o
Read, 2011[71]	Australia	7/2004 – 7/2011	No	Yes	From 2007/2008–2010/2011, incidence decreased in MSW from 22.9% to 2.9% and from 16.1% to 11.7% in 21-29 y/o. No change was observed in MSM

-Abbreviations: ref: reference; y/o: year/s old; MSW: men who have sex with women; MSM: men who have sex with men; vax: vaccine or vaccinated
- Unless specified, pre-vaccine period is defined as any time before 31 December 2006
- Unless specified, post-vaccine period is defined as any after 1 January 2007
- Unless specified, all results are statistically significant at <0.05

2.6. References

1. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: human papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007:1-636.

2. Weinstock H, Berman S, Cates Jr W. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Reprod Health 2004;36:6-10.

3. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890-907.

4. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.

5. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. J Natl Cancer Inst 1995;87:796-802.

6. Muñoz N, Bosch FX, De Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.

7. Cogliano V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6:204.

8. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.

9. Bosch FX, de Sanjosé S. Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. J Natl Cancer Inst Monogr 2003:3-13.

10. Muñoz N, Bosch FX, Chichareon S. A multinational case-control study on the risk of cervical cancer linked to 25 HPV types: Which are the high-risk types? 18th International Papillomavirus Conference 2000:125.

11. J. Ferlay, I. Soerjomataram, R. Dikshit, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136:E359-E386.

12. Franceschi S, Denny L, Irwin KL, et al. Eurogin 2010 roadmap on cervical cancer prevention. Int J Cancer 2011;128:2765-74.

13. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. (Accessed November 4, 2013, at http://globocan.iarc.fr.)

14. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. Br J Cancer 2003;88:63-9.

15. Bosch FX, Burchell AN, Schiffman M, et al. Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 2008;26:K1-K16.

16. Bulkmans NWJ, Bleeker MCG, Berkhof J, Voorhorst FJ, Snijders PJF, Meijer CJLM. Prevalence of types 16 and 33 is increased in high-risk human papillomavirus positive women with cervical intraepithelial neoplasia grade 2 or worse. Int J Cancer 2005;117:177-81.

17. Castle PE, Solomon D, Schiffman M, Wheeler CM. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J Natl Cancer Inst 2005;97:1066-71.

18. Khan MJ, Castle PE, Lorincz AT, et al. The elevated 10-Year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005;97:1072-9.

19. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76-84.

20. Van Hamont D, Van Ham MAPC, Bakkers JMJE, Massuger LFAG, Melchers WJG. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006;44:3122-9.

21. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol 1995;33:2058-63.

22. Dinh TH, Sternberg M, Dunne EF, Markowitz LE. Genital warts among 18- to 59-year-olds in the United States, National Health and Nutrition Examination Survey, 1999-2004. Sex Transm Dis 2008;35:357-60.

23. Kjær SK, Tran TN, Sparen P, et al. The burden of genital warts: A study of nearly 70,000 women from the general female population in the 4 Nordic countries. J Infect Dis 2007;196:1447-54.

24. Pirotta M, Ung L, Stein A, et al. The psychosocial burden of human papillomavirus related disease and screening interventions. Sex Transm Infect 2009;85:508-13.

25. Hu D, Goldie S. The economic burden of noncervical human papillomavirus disease in the United States. Am J Obstet Gynecol 2008;198:500.e1-.e7.

26. Dempsey AF, Koutsky LA. National burden of genital warts: A first step in defining the problem. Sex Transm Dis 2008;35:361-2.

27. Donovan B, Franklin N, Guy R, et al. Quadrivalent human papillomavirus vaccination and trends in genital warts in Australia: Analysis of national sentinel surveillance data. Lancet Infect Dis 2011;11:39-44.

28. Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2007;56:1-24.

29. FDA licensure of bivalent human papillomavirus vaccine (HPV2, cervarix) for use in females and updated HPV vaccination recommendations from the advisory committee on immunization practices (ACIP). MMWR 2010;59:626-9.

30. CDC. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the advisory committee on immunization practices (ACIP). MMWR 2010;59:630-2.

31. Highlights of prescribing information. Gardasil [human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine]. US Food Drug Administration, 2011. (Accessed January 28, 2013, at http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ ApprovedProducts/UCM111263.pdf.)

32. Dorell CG, Yankey D, Santibanez TA, Markowitz LE. Human papillomavirus vaccination series initiation and completion, 2008-2009. Pediatrics 2011;128:830-9.

33. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.

34. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374:301-14.

35. Dillner J, Kjaer SK, Wheeler CM. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: Randomised controlled trial. BMJ 2010;341:c3493.

36. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med 2011;364:401-11.

37. Petrosky E, Bocchini J.A, Jr., Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: Updated hpv vaccination recommendations of the advisory committee on immunization practices. MMWR 2015;64:300-4.

38. Joura EA, Giuliano AR, Iversen O-E, et al. A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women. N Engl J Med 2015;372:711-23.

39. Brown DR, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; Types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. J Infect Dis 2009;199:926-35.

40. Wheeler CM, Castellsague X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012;13:100-10.

41. Wheeler CM, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16-26 years. J Infect Dis 2009;199:936-44.

42. Obaro SK, Adegbola RA, Banya WS, Greenwood BM. Carriage of pneumococci after pneumococcal vaccination. Lancet 1996;348:271-2.

43. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001;344:403-9.

44. Pai R, Moore MR, Pilishvili T, et al. Postvaccine genetic structure of Streptococcus pneumoniae serotype 19A from children in the United States. J Infect Dis 2005;192:1988-95.

45. Huang SS, Platt R, Rifas-Shiman SL, et al. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics 2005;116:e408-e13.

46. Gonzalez BE, Hulten KG, Lamberth L, Kaplan SL, Mason Jr EO. Streptococcus pneumoniae serogroups 15 and 33: An increasing cause of pneumococcal infections in children in the United States after the introduction of the pneumococcal 7-valent conjugate vaccine. Pediatr Infect Dis J 2006;25:301-5.

47. Franco EL, Cuzick J. Cervical cancer screening following prophylactic human papillomavirus vaccination. Vaccine 2008;26 Suppl 1:A16-23.

48. Kahn JA. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med 2009;361:271-834.

49. Brotherton JML. HPV prophylactic vaccines: Lessons learned from 10 years experience. Future Virol 2015;10:999-1009.

50. Dorell C, Stokley S, Yankey D, Jeyarajah J, MacNeil J, Markowitz L. National and state vaccination coverage among adolescents aged 13-17 years - United States, 2011. MMWR 2012;61:671-7.

51. CDC. Human papillomavirus vaccination coverage among adolescent girls, 2007-2012, and postlicensure vaccine safety monitoring, 2006-2013 - United States. MMWR 2013;62:591-5.

52. Frieden T. Doctors: Are you helping reduce HPV? The Blog: The Huffington Post; 2013.

53. Poulsen S. The Danish HPV programme: A success story—vaccination by general practitioners. Presented at: The Eurogin 2011 International Congress [abstract SS4-11]; 2011; Lisbon.

54. Mikolajczyk RT, Kraut AA, Horn J, Schulze-Rath R, Garbe E. Changes in incidence of anogenital warts diagnoses after the introduction of human papillomavirus vaccination in Germany-an ecologic study. Sex Transm Dis 2013;40:28-31.

55. Hense S, Hillebrand K, Horn J, et al. HPV vaccine uptake after introduction of the vaccine in Germany: An analysis of administrative data. Hum Vaccin Immunother 2014;10:1729-33.

56. Garland SM, Skinner SR, Brotherton JM. Adolescent and young adult HPV vaccination in Australia: achievements and challenges. Prev Med 2011;53:S29-35.

57. Agius PA, Pitts MK, Smith AMA, Mitchell A. Human papillomavirus and cervical cancer: Gardasil® vaccination status and knowledge amongst a nationally representative sample of Australian secondary school students. Vaccine 2010;28:4416-22.

58. Weisberg E, Bateson D, McCaffery K, Skinner SR. HPV vaccination catch up program: Utilisation by young Australian women. Aust Fam Physician 2009;38:72-6.

59. Hariri S, Markowitz L. Monitoring HPV vaccine impact: Early results and ongoing challenges. J Infect Dis 2012;206:1633-5.

60. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. Ann Intern Med 2009;151:65-94.

61. Brotherton JML, Fridman M, May CL, et al. Early effect of the HPV vaccination programme on cervical abnormalities in Victoria, Australia: An ecological study. Lancet 2011;377:2058-92.

62. Powell SE, Hariri S, Steinau M, et al. Impact of human papillomavirus (HPV) vaccination on HPV 16/18-related prevalence in precancerous cervical lesions. Vaccine 2012;31:109-13.

63. Baandrup L, Blomberg M, Dehlendorff C, et al. Significant decrease in the incidence of genital warts in young Danish women after implementation of a national human papillomavirus vaccination program. Sex Transm Dis 2013;40:130-5.

64. Bauer HM, Wright G, Chow J. Evidence of human papillomavirus vaccine effectiveness in reducing genital warts: An analysis of California public family planning administrative claims data, 2007-2010. Am J Public Health 2012;102:833-5.

65. Blomberg M, Dehlendorff C, Munk C, Kjaer SK. Strongly decreased risk of genital warts after vaccination against human papillomavirus: nationwide follow-up of vaccinated and unvaccinated girls in Denmark. Clin Infect Dis 2013;57:929-34.

66. Fairley CK, Hocking JS, Gurrin LC, et al. Rapid decline in presentations of genital warts after the implementation of a national quadrivalent human papillomavirus vaccination programme for young women. Sex Transm Infect 2009;85:499-502.

67. Flagg EW, Schwartz R, Weinstock H. Prevalence of anogenital warts among participants in private health plans in the United States, 2003-2010: Potential impact of human papillomavirus vaccination. Am J Public Health 2013;103:1428-35.

68. Leval A, Herweijer E, Arnheim-Dahlstrom L, et al. Incidence of genital warts in Sweden before and after quadrivalent human papillomavirus vaccine availability. J Infect Dis 2012;206:860-6.

69. Nsouli-Maktabi H, Ludwig SL, Yerubandi UD, Gaydos JC. Incidence of genital warts among U.S. service members before and after the introduction of the quadrivalent human papillomavirus vaccine. MSMR 2013;20:17-20.

70. Oliphant J, Perkins N. Impact of the human papillomavirus (HPV) vaccine on genital wart diagnoses at Auckland Sexual Health Services. N Z Med J 2011;124:51-8.

71. Read TRH, Hocking JS, Chen MY, et al. The near disappearance of genital warts in young women 4 years after commencing a national human papillomavirus (HPV) vaccination programme. Sex Transm Infect 2011;87:544-7.

72. Cummings T, Zimet GD, Brown D, et al. Reduction of HPV infections through vaccination among at-risk urban adolescents. Vaccine 2012;30:5496-9.

73. Kahn JA, Brown DR, Ding L, et al. Vaccine-type human papillomavirus and evidence of herd protection after vaccine introduction. Pediatrics 2012;130: e249-e256.

74. Markowitz LE, Hariri S, Lin C, et al. Reduction in human papillomavirus (HPV) prevalence among young women following hpv vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013; 208:385-393.

75. Tabrizi SN, Brotherton JM, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645-51.

76. Bogaards JA, Coupe VMH, Xiridou M, et al. Long-term impact of human papillomavirus vaccination on infection rates, cervical abnormalities, and cancer incidence. Epidemiology 2011;22:505-15.

77. Choi YH, Jit M, Gay N, et al. Transmission dynamic modelling of the impact of human papillomavirus vaccination in the United Kingdom. Vaccine 2010;28:4091-102.

78. Cuzick J, Castanon A, Sasieni P. Predicted impact of vaccination against human papillomavirus 16/18 on cancer incidence and cervical abnormalities in women aged 20-29 in the UK. Br J Cancer 2010;102:933-9.

79. Drolet M, Boily MC, Van de Velde N, Franco EL, Brisson M. Vaccinating girls and boys with different human papillomavirus vaccines: Can it optimise population-level effectiveness? PloS One 2013;8:e67072.

80. Elbasha EH, Dasbach EJ, Insinga RP. Model for assessing human papillomavirus vaccination strategies. Emerg Infect Dis 2007;13:28-41.

81. Gauthier A, Martin-Escudero V, Moore L, et al. Long-term clinical impact of introducing a human papillomavirus 16/18 AS04 adjuvant cervical cancer vaccine in Spain. Eur J Public Health 2008;18:674-80.

82. Goldie SJ, Grima D, Kohli M, et al. A comprehensive natural history model of HPV infection and cervical cancer to estimate the clinical impact of a prophylactic HPV-16/18 vaccine. Int J Cancer 2003;106:896-904.

83. Kohli M, Ferko N, Martin A, et al. Estimating the long-term impact of a prophylactic human papillomavirus 16/18 vaccine on the burden of cervical cancer in the UK. Br J Cancer 2007;96:143-50.

84. Hughes JP, Garnett GP, Koutsky L. The theoretical population-level impact of a prophylactic human papilloma virus vaccine. Epidemiology 2002;13:631-9.

85. Marty R, Roze S, Bresse X, Largeron N, Smith-Palmer J. Estimating the clinical benefits of vaccinating boys and girls against HPV-related diseases in Europe. BMC Cancer 2013;13:10.

86. Smith MA, Canfell K, Brotherton JML, Lew JB, Barnabas RV. The predicted impact of vaccination on human papillomavirus infections in Australia. Int J Cancer 2008;123:1854-63.

87. Smith MA, Lew JB, Walker RJ, et al. The predicted impact of HPV vaccination on male infections and male HPV-related cancers in Australia. Vaccine 2011;29:9112-22.

88. Van De Velde N, Boily MC, Drolet M, et al. Population-level impact of the bivalent, quadrivalent, and nonavalent human papillomavirus vaccines: A model-based analysis. J Natl Cancer Inst 2012;104:1712-23.

89. Vanska S, Auranen K, Leino T, et al. Impact of vaccination on 14 high-risk HPV type infections: a mathematical modelling approach. PloS One 2013;8:e72088.

90. Van de Velde N, Brisson M, Boily MC. Understanding differences in predictions of HPV vaccine effectiveness: A comparative model-based analysis. Vaccine 2010;28:5473-84.

91. Woodman CB, Collins SI, Young LS. The natural history of cervical HPV infection: unresolved issues. Nat Rev Cancer 2007;7:11-22.

92. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191:731-8.

93. Winer RL, Koutsky LA. Human papillomavirus through the ages. J Infect Dis 2005;191:1787-9.

94. Moscicki AB, Shiboski S, Broering J, et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. J Pediatr 1998;132:277-84.

95. Franco EL, Rohan TE, Villa LL. Epidemiologic evidence and human papillomavirus infection as a necessary cause of cervical cancer. J Natl Cancer Inst 1999;91:506-11.

96. Hong K, Greer CE, Ketter N, Van Nest G, Paliard X. Isolation and characterization of human papillomavirus type 6-specific T cells infiltrating genital warts. J Virol 1997;71:6427-32.

97. Tagami H, Oku T, Iwatsuki K. Primary tissue culture of spontaneously regressing flat warts. In vitro attack by mononuclear cells against wart-derived epidermal cells. Cancer 1985;55:2437-41.

98. Beutner KR, Wiley DJ, Douglas JM, et al. Genital warts and their treatment. Clin Infect Dis 1999;28:S37-S56.

99. Stone KM. Human papillomavirus infection and genital warts: Update on epidemiology and treatment. Clin Infect Dis 1995;20:S91-S7.

100. Romanowski B, Schwarz TF, Ferguson LM, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: Results from a randomized study. Hum Vaccines 2011;7:1374-86.

101. Dobson SRM, McNeil S, Dionne M, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: A randomized clinical trial. JAMA 2013;309:1793-802.

102. Groner JA, Harris GD, Harper DM. Reduction in HPV prevalence—No evidence to support HPV vaccination reduces HPV prevalence. J Infect Dis 2014;209:1302-4.

103. Hariri S, Markowitz LE, Dunne EF, Unger ER. Population impact of HPV vaccines: Summary of early evidence. J Adolesc Health 2013;53:679-82.

3.0 Chapter 3:

Temporal trends in vaccine-type HPV genotypic prevalence between pre- and post-vaccine periods (2003-2010)

Abstract

The present ecological study aims to investigate early virologic impact of HPV vaccination at the population level, namely temporal trends in vaccine-type HPV prevalence (HPV 6, 11, 16, and 18) before and after commercial availability of the quadrivalent vaccine, Gardasil, in 2006 in the U.S. Data were drawn from more than 8,000 females aged 14-59 years enrolled between 2003-2010 in the NHANES HPV Vaginal Swab Surveys, a population-based, cross-sectional survey collecting HPV DNA specimens as well as socio-demographic and sexual behavior information. Vaccine-type HPV genotypic prevalence was compared between females from the first two 2-year surveys (2003-2004 and 2005-2006; the "pre-vaccine period") and females from the latter two 2-year surveys (2007-2008 and 2009-2010; the "post-vaccine period") with specific analyses that stratified by age groups, states aggregated by highest and lowest vaccine coverage, and prior vaccination history. "Modified Poisson" regression models with adjusted prevalence ratios were used to compare vaccine-type HPV prevalence between vaccine periods, and GEE regression models were employed to determine risk factors associated with vaccine-type HPV infection. In the entire sample, prevalence of HPV 6 decreased between the two periods from 2.8% (95% CI: 2.2-3.4) to 1.8% (95% CI: 1.4-2.3). Post-vaccine period females aged 14-19 years had a 73% and 51% reduction in LR and HR-HPV, respectively. LR vaccine-type HPV prevalence was significantly less in vaccinated females compared to those unvaccinated. No significant HPV prevalence difference was found between aggregated states with high versus low vaccine coverage. These findings of decreased vaccine-type HPV prevalence in adolescent females should be used by public health professionals and pediatricians to help educate parents on the virologic activity and benefits of HPV vaccination for their children.

3.1. Introduction

Human papillomavirus (HPV) is a small double-stranded DNA virus which infects cutaneous and mucosal epithelial cells.[1] HPV-infected epithelial cells undergo terminal differentiation encoding eight open reading frames (ORFs) which are transcribed as polycystic mRNAs from a single DNA strand in order to override any normal regulation of differentiation to produce progeny virions. HPV is tissue-tropic and its replication depends on squamous epithelial differentiation.[2]

Of the approximately 120 HPV types that have been identified, 40 types sort into 15 alpha species which infect the genital tract.[3] These 40 HPV types are divided into two groups: high-risk (HR), also referred to as oncogenic or carcinogenic, and low-risk (LR). HR HPV genotypes have been etiologically linked to invasive cervical cancer (ICC)[4,5] as well as vaginal, vulvar, penile, anal, and a subset of head and neck cancers.[6-10]

HPV is by far the most common sexually transmitted infection in the U.S. with approximately 6.2 million individuals infected yearly.[11] Sexual activity—often within the first 6 months of sexual debut—is the most pronounced risk factor for incident HPV infection in a female's genital tract.[12,13] HPV infection is age-dependent[14] and detected most often in sexually-active females aged 20-24 years.[15]

ICC is the fourth most common cancer in U.S. females, with approximately 530,000 cases diagnosed yearly.[16] It is, however, the number one cancer in sub-Saharan African and Southeast Asian females because high quality screening and early and effective treatment are

sparse to non-existent.[17] The World Health Organization (WHO) estimates that ICC is responsible for approximately 270,000 deaths worldwide—namely developing countries.[16]

The WHO's International Agency for Research (IARC) has periodically classified and categorized 20 HPV genotypes in varying degrees of high-risk based on their potential carcinogenicity.[18-20] Twelve genotypes, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, fall into Group 1 because of "sufficient evidence for cervical cancer;" HPV 68 is in Group 2A because it is "probably carcinogenic;" and the remaining seven genotypes, HPV 26, 53, 66, 67, 70, 73, 82, are in Group 2B and considered "possibly carcinogenic." [20]

HPV 16 and 18 are overwhelmingly the HR types most strongly associated with approximately 70% of ICC.[21,22] HPV 18, however, is most commonly detected in adenocarcinoma of the cervix.[22,23] Approximately 40% of women who repeatedly test HPV positive (referred to as "persistent") for type-16 will go on to develop high-grade (precancerous) cervical intraepithelial neoplasia (CIN).[24] Moreover, there is a twelvefold increase in the risk of developing high-grade CIN in females with persistent HPV 16 and/or 18 infection compared to other HR HPV genotypes.[25] Stage-3 CIN left untreated for many years can invade the base membrane of the epithelium causing frank cervical cancer.[26,27]

The 20 HPV types classified as LR HPV include HPV 6, 11, 32, 40, 42, 43, 44, 54, 55, 61, 62, 64, 71, 72, 74, 81, 83, 84, 87, and 89.[28] LR HPV 6 and 11 are associated with 90% of penile, vaginal, and anal warts.[29] Genital warts (GWs) are often the immediate clinical manifestation of incident HPV infection.[30] While GWs are easily treatable in an out-patient setting, the psychological burden caused by GWs, as well as the annual cost to health care systems, should not be underestimated.[31,32]

HPV prophylactic vaccination against HPV 6, 11, 16, and 18 has existed in the U.S. since 2006. Routine vaccination is recommended for all individuals aged 11 or 12 years. Catch–up vaccination is recommended for females and men who have sex with men aged 13 through 26 years, and up to 21 years for men who have sex with women.[33-35] The three U.S. Food and Drug Administration (FDA)-approved, commercially available vaccines differ in the number of specific HPV genotypes they prevent; however, all target HPV 16 and 18. Pivotal studies of the three vaccines reported 90-98% vaccine efficacy on all endpoints (e.g., CIN 2/3 or GWs) and all documented an excellent safety profile.[36,37 38,39]

While long-term follow-up HPV vaccine efficacy data have been published on females enrolled in the initial industry-sponsored HPV vaccine studies, it exists only for those who received the vaccine, not placebo.[40,41] Moreover, many of the initial vaccine randomized controlled trials (RCTs) enrolled a select female patient population due to strict inclusion and exclusion criteria, such as limiting the number of lifetime sexual partners and absence of HPV DNA at entry. Thus, RCT data, regardless of follow-up duration, are unable to offer evidence of decreased HPV DNA prevalence at the population level between the pre- and post-vaccine eras. This is only possible from population-based, cross-sectional survey data of female HPV DNA prevalence before and after the advent of widespread HPV vaccination in 2006.

With a median age of 49 years for ICC in the U.S.,[42] it will be approximately 25 years until significant clinical benefit of HPV vaccination—namely, a reduction in ICC incidence—can be established. Monitoring temporal trends in HR vaccine-type HPV 16 and 18 prevalence, as well as LR vaccine-type HPV 6 and 11, is the surest and most expeditious way of monitoring early vaccine impact at the population level.[43] Marked decreases in the virologic prevalence of these vaccine-specific HPV genotypes in female populations where vaccination is widespread

should consequentially result in declined incidence of GWs and cervical abnormalities, and over time, ICC.

The U.S. CDC-sponsored National Health and Nutrition Examination Survey (NHANES) has collected and analyzed HPV DNA vaginal swab samples on over 8,000 females between 2003 and 2010. NHANES vaginal swab survey data are ideal for capturing temporal trends in HPV genotypic prevalence. Combining the first two 2-year surveys—2003-2004 and 2005-2006—can serve as a pre-vaccine era period of unvaccinated/unexposed females, while combining the latter two 2-year surveys—2007-2008 and 2009-2010—serves as the post-vaccine era period representing the general U.S. female population during the first four years of widespread HPV vaccination. No other population-based cross-sectional survey combines socio-demographic information and sexual activity behavioral with laboratory data from both the pre- and post-vaccine eras.

Besides temporal trends analyses comparing HPV genotypic prevalence between the pre- and post-vaccine era NHANES, I will attempt to determine if there are geographical differences in vaccine-type HPV based on vaccine uptake. I hypothesize in this analysis that the top-ten states with the highest vaccine coverage will show a larger decrease in vaccine-type HPV prevalence (HPV 16 and/or 18) in the post-vaccine period (2007-2010) than the bottom-ten states with the least vaccine coverage. This would be another early indication that the vaccine is doing what it is supposed to. This analysis is novel in that no published data exist on the correlation of HPV prevalence with HPV vaccination rates in the U.S.

Unlike previously published ecological studies examining temporal trends in vaccine-type HPV genotypic prevalence in young females aged 14 to 19 years,[44-46] my investigations

include females up to age 59 years. I also examine whether socio-demographic characteristics such as age, race/ethnicity, and education as well as other known HPV risk factors, including sexual activity and marital status,[12-14,47] confound prevalence estimate comparisons.

3.2 Methods

Study and collection of data

NHANES is the largest of the four major CDC-sponsored National Center for Health Statistics (NCHS) data collection programs. The initial basis for NCHS surveys was the National Health Survey Act (P.L.84–652), enacted on July 3, 1956.[48]

The NHANES program commenced in the early 1960s, conducting a series of surveys focusing on different population groups and health topics. It became a continuous survey program in 1999 that focused on a variety of health and nutrition measurements of a nationally representative sample of approximately 20,000 persons each year. NHANES obtains a nationally representative sample of non-institutionalized U.S. individuals by using a complex, stratified, multistage probability sample design with unequal probabilities.[49] Adolescents, non-Hispanic blacks, and Mexican Americans are oversampled in order to allow sufficient sizes for subgroup analysis.

HPV DNA testing for females was added to NHANES in its 2003-2004 survey.[47] HPV DNA testing occurred on females aged 14-59 years with the use of self-collected vaginal swabs. Consenting participants have a household interview followed by a physical examination in a mobile examination center (MEC). MECs are made up of four sideways-linked trailers (similar

to a mobile home) which resemble a fully-functioning medical clinical with myriad diagnostic equipment (e.g., CT scan), and banks of computers for data entry. They are situated in locations convenient for enrolled participants.

Sample for analysis

In each of the four cycles (2003-2004; 2005-2006; 2007-2008; 2009-2010), the CDC's NCHS enrolled approximately 2,500 females for its NHANES HPV Vaginal Swab Surveys. Final data available for HPV DNA analysis usually drops to 2,100 females for a number of reasons, including 1) refusal of examination in the MEC; 2) unwillingness to self-collect a cervicovaginal swab sample; and 3) inadequacy of samples for DNA typing. Data from 9, 850 females aged 14-59 years are available for my analysis from the four 2-year NHANES HPV Vaginal Swab Surveys. All data are publicly available for downloading on the CDC's NHANES website, except for those pertaining to female minors aged 14-17 years. Data on minors, termed "limited use data," are only available for analyses after a research proposal has been approved by the NCHS.

Specimen collection

The protocol detailing instructions and methods of specimen collection has been described elsewhere.[47] In brief, self-collection of a cervicovaginal sample took place in private in the bathroom of a MEC. Females were given a collection device that had a small foam swab on a plastic handle packaged in an individual resealable plastic sleeve (Catch-All Sample Collection Swabs Epicenter, Madison, WI). Foam swabs were to be inserted into the vagina—similar to inserting a tampon—gently turned for 10 seconds and then replaced into the plastic

sleeve. NHANES personnel collected the material and mailed it to a CDC laboratory for processing. Detailed specimen processing instructions are outlined in the NHANES Laboratory Procedure Manual.

Laboratory methods

DNA extractions were performed within one month of collecting samples employing a modified QIAmp Mini Kit.[47,50] The extract (100 µL total volume) was either tested immediately or stored at –20C. To serve as a contamination control, a water blank was processed through all steps of extraction for every 40 samples.

DNA from the vaginal swab was extracted using two assays, the Qiagen Hybrid Capture (HC2) and Roche Linear Array (LA). HC2 is a nucleic acid hybridization microplate assay with signal amplification. It uses chemiluminescence for the qualitative detection of eighteen types of HPV DNA in cervical specimens. The HC2 dichotomously differentiates between the two HPV DNA groups: low-risk (LR) HPV Types: 6, 11, 42, 43, and 44; and high-risk (HR) HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. It is, however, unable to determine the "specific" HPV type present.

The Roche LA is based on HPV L1 consensus polymerase chain reaction (PCR) with biotinylated PGMY09/11 primer sets. It also includes biotinylated β-globin primers as an internal control for sample amplification. The primer mix amplifies essentially all HPV types that are found in the genital tract along with the human β-globin.[51] All samples are hybridized to the typing strip which included probes for 37 HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39. The Roche LA is considered "research only" because it has not been approved by

the FDA for commercial use in the U.S. It is, however, approved for commercial use in the European Union.

The 2003-2004 NHANES Vaginal Swab Survey initially analyzed specimens using the Roche prototype line blot assay. The second and following NHANES Surveys discontinued the Roche prototype line blot assay and employed the Roche Linear Array Assay (LA). Saved samples from the 2003-2004 Survey were re-analyzed with the Roche LA, thus allowing for analyses of four 2-year surveys using the same laboratory methods.

Demographic and behavioral data of study subjects

Before all female participants aged 14-59 years enter the MEC to self-collect vaginal swab samples, household interviews are conducted to obtain demographic information, including age, race, ethnicity, education, and marital status. Race and ethnicity were self-reported into categories, including non-Hispanic black, non-Hispanic white, and Mexican American. Using audio computer-assisted self-interview (ACASI), participants self-reported sexual history information. Sex was defined as vaginal, oral, or anal sex. For those females aged 14 to 17 years who reported sexual activity, additional questions about sexual behavior were asked, such as age at first sex and number of lifetime sex partners. For the females aged 18 years or older who reported past sexual activity, they were asked additional questions on the number and gender of sex partners in the last 12 months, lifetime sex partners, and past history of sexually transmitted infections.

HPV prevalence data aggregated by state

To determine if there are geographical differences in vaccine-type HR HPV prevalence based on vaccine uptake, I compare the prevalence of HPV 16 and/or 18 in the top-10 states with the highest vaccination coverage with that of the bottom-10 states with the lowest vaccination coverage in the post-vaccine period (2007-2010). To ascertain if the prevalence change in HPV 16 and 18 genotypes in the post-vaccine era is novel, I compare the prevalence in the aggregated states from the first two 2-year pre-vaccine era surveys to serve as a baseline.

States have been categorized based on published data from the adolescent portion of the CDC-sponsored National Immunization Survey, NIS-Teen.[52] NIS-Teen is considered the most accurate of all immunization surveys because of its use of physician verification.[53] The top-10 states have a median of 44.8% HPV vaccine coverage for all 3 doses while the bottom-10 states have a median of 22.5%. The top-10 states in descending order of most HPV vaccine coverage are: Rhode Island; South Dakota; Massachusetts; Connecticut; Washington; Wisconsin; Nebraska; New Hampshire; Pennsylvania; Virginia. The bottom-10 states in ascending order with least vaccine coverage are: Idaho; Arkansas; Mississippi; Alabama; Utah; Georgia; Indiana; Florida; Alaska; Kansas. (See **Figures 3.1a & 3.1b** for details.)

Categorization of confounding variables

Potential confounders to be included in the pre-vaccination/post-vaccination analyses are: 1) age; 2) race/ethnicity; 3) education; 4) marital status; 5) ever having sex; 6) age at sexual debut; and 7) total number of lifetime male sexual partners. Age is categorized as: 14-19 years; 20-24 years; 25-29 years; 30-39 years; 40-49 years; and 50-59 years. Age at sexual debut is

categorized as younger than 16 years of age, older than 16 years of age, or never having had sex. (See **Table 3.1** for a detailed listing of variables and their categorizations.)

Statistical Analysis

All analyses were conducted using NHANES 2003-2010 sample weights to account for the complex survey design and to provide unbiased estimates of HPV prevalence and predictors for the total US population.[54,55] Four-year weights are provided and recommended by NHANES when combining two 2-year surveys. Data already account for weighting and a complex sample design, where WTMEC4YR = ½ x WTMEC2YR for a 4-year sample.

Since the sample weights in NHANES were employed to oversample certain groups in an attempt to match the demographics of the U.S., I adjusted for strata and cluster to create unbiased estimates of my standard errors (e.g., 95% confidence intervals and p-values). The primary analyses compare vaccine-type HPV genotypic prevalence in females in the pre-vaccine period (2003-2006) and post-vaccine period (2007-2010) samples across the 50 U.S. states, with the latter being further stratified into two groups based on the aggregated ten states with the highest and least vaccination coverage in the post-vaccine era as per CDC-sponsored NIS-Teen Survey data.

Prevalence estimates of HPV DNA will be reported as percentages with 95% confidence intervals. Confidence intervals for HPV type-specific prevalence were estimated using methods adopted by Korn and Graubarde for proportions with small expected number of positive counts in complex multicenter survey data.[56]

To assess for differences in proportions of females testing HPV DNA-positive, $\chi 2$ tests and student t-tests were used. The student t-test assumes that the data has a normal distribution, and that the covariance is small. The CDC's National Center for Health Statistics (NCHS) encourages use of the student t-test for NHANES data to detect differences in health outcomes or risk factors between subpopulations, and it contends that NHANES data "do meet both assumptions" provided data are not divided into very small sub-domains. To explore for confounding, bivariate analyses with demographic and behavioral characteristics were conducted using a survey design-adjusted Wald F test.

I employ "modified Poisson" regression, as outlined by Zou,[57] to adjust comparisons of prevalence by socio-demographic characteristics and sexual activity behavior that were found to vary between the groups of females and report adjusted prevalence ratios (aPR). Modified Poisson regression for binary outcomes in population surveys provides robust error variance estimation and creates 95% confidence intervals with the correct coverage.[58] Moreover, the robust error variances can be attained by using the repeated statement along with the subject identifier even when there is only one observation per subject. Statistical significance was tested at the level of $P< .05$. Associations were considered significant if the P value for the Satterthwaite adjusted F test[59] is <0.05, and those variables were retained in the main effects model. The formula for Satterthwaite's approximation for the degrees of freedom for the approximate t statistic is: df = [((w1+w2)2)/(([(w12)/(n1-1)]+[(w22)/(n2-1)]))].[60] All analyses were be conducted using SAS version 9.4.[61]

3.3 Results

Comparison of HPV Prevalence between 2003–2006 and 2007–2010

A total of 9,850 females between the ages of 14-59 years were included in the analyses: 4,990 from the first two 2-year surveys of 2003-2004 and 2005-2006 (henceforth referred to as the pre-vaccine period) and 4,860 from the latter two 2-year surveys of 2007-2008 and 2009-2010 (henceforth referred to as the post-vaccine period). **Table 3.2** documents the age distribution of females enrolled in the pre- and post-vaccine periods. Due to NHANES's discontinuation of over-sampling younger females after the 2005-2006 Vaginal Swab Survey, the most significant demographic difference between females in the two periods is the approximate 50% reduction in the number of females aged 14-19 years. In this age group, there were 1,660 pre-vaccine period females compared to 887 in the post-vaccine period. However, the weighted distribution of females in this age category is not significantly different: 13.4% compared to 13.0%.

Table 3.3a details the difference in HPV prevalence among the entire sample of females between the pre- and post-vaccine periods. No significant unadjusted or adjusted difference was observed for all HPV (37 HPV genotypes types combined), HPV vaccine types (HPV 6, 11, 16, and 18 combined), or HR HPV vaccine types (16 and/or 18). LR vaccine-type (HPV 6 and/or 11) prevalence decreased in the post-vaccine period from 2.8% to 1.7% with an unadjusted prevalence ratio (PR) of 0.63 (95% CI: 0.46-0.87). After adjusting for socio-demographic variables and sexual behavior, this decreased prevalence no longer remained statistically significant (aPR = 0.81; 95% CI: 0.55-1.19).

To ascertain if there was an increased or decreased trend in HR vaccine-type prevalence before the advent of HPV vaccination, the two 2-year surveys from the pre-vaccine period were compared. As documented in **Table 3.3b**, no statistically significant difference in HR vaccine-type prevalence was observed between the 2003-2004 and 2005-2006 surveys.

When stratified by age, as documented in **Table 3.4,** decrease in HPV genotypic prevalence was only observed in post-vaccine period females from the youngest age group. In adolescents aged 14-19 years, all HPV decreased from 27.5% (95% CI: 24.7-30.4) to 21.5% (18.2-24.8) with a PR of 0.78; (95% CI: 0.66-0.94). LR vaccine-type prevalence decreased by 73%, from 8.1% (95% CI: 5.0-11.3) to 2.2% (0.79-3.6) with a PR of 0.27 (95% CI: 0.13-0.56). And, HR vaccine-type prevalence decreased by 51%, from 6.0% (95% CI: 4.5-7.5) to 2.9% (95% CI: 1.9-4.0) with a PR of 0.49 (95% CI: 0.32-0.76).

Comparison of individual HPV genotypic weighted prevalence between vaccine periods

As documented in **Tables 3.5a-3.5f,** the only change in individual genotypic prevalence in the entire sample of females was a decrease of HPV 6 prevalence in the post vaccine period: 2.8% (95% CI: 2.2-3.4) compared to 1.8% (95% CI: 1.4-2.3). When stratified by age, decreased prevalence of individual vaccine-type genotypes was only observed in the youngest group of females aged 14-19 years. In this age group, decreased prevalence was found for HPV 6 (5.3% vs. 1.6%) and HPV 16 (5.8% vs. 3.0%). There was a trend towards decrease prevalence in HPV 18 (1.6% vs. 1.0%); however, with so few observations in this particular age group, 95% confidence intervals considerably overlapped (0.9-2.3 vs. 0.2-1.7).

Weighted HPV prevalence among females ages 14-29 years according to vaccination status

Self-reported vaccine history data exists on 1,835 of females aged 14-29 years enrolled in the post-vaccine period NHANES Vaginal Swab Studies. NHANES defines "vaccinated" as receiving ≥1 dose of either the bivalent or quadrivalent HPV vaccine. As documented in **Table 3.6**, an 85% reduction in vaccine-type LR HPV prevalence was observed in 14-29 year-old vaccinated females: 3.5% in the unvaccinated versus 0.5% in the vaccinated (PR = 0.15; 95% CI: 0-0.51). A greater than 50% reduction in vaccine-type HR HPV prevalence was documented in vaccinated females with a PR of 0.42 (95% CI 0-1.18); however, it was not statistically significant. It is important to note that for both observations, the prevalence estimates had a relative standard error ≥30%, and thus, these results are considered unstable and should be interpreted with caution.

Geographical analysis of HR HPV prevalence in states with high-and low-vaccine coverage

Figures 3.1a and 3.1b detail 2010 NIS-Teen Survey data of the top-10 states with the highest reported HPV vaccine uptake—defined as receiving all 3 recommended doses—and the bottom-10 states with the lowest reported vaccine coverage. The top-10 states had a median 44.8% coverage (range: 41.5%-55.1%), while the bottom-10 states had a median 22.5% coverage (range: 17.6%-25.1%).

The aggregated states were compared in the 4-year pre-vaccine period in order to determine baseline vaccine-type HR HPV prevalence before the advent of widespread HPV vaccination in 2006. **Table 3.7a** illustrates a slight, non-statistically significant decrease in

vaccine-type HR HPV prevalence in females residing in the top-10 states with the highest reported vaccine coverage: 5.7% versus 6.4% (p=0.68). **Table 3.7b** documents that vaccine-type HR HPV prevalence decreased modestly in both sets of aggregated states in the post-vaccine period. Nevertheless, the slight decrease observed in vaccine-type HR HPV prevalence in females residing in the top-10 compared to the bottom-10 states—4.5% compared to 5.9% (p=0.38)—did not reach statistical significance.

Risk factors associated with vaccine-type HPV prevalence in all females from both periods

Tables 3.8a documents the demographic and sexual behavior variables which were associated with combined LR and HR vaccine-type HPV prevalence in all females from both the pre- and post-vaccine periods. In the multi-level GEE model, increased risk of vaccine-type HPV was associated with younger age—observed in all categorized female age groups less than 40 years; lower education status (e.g., less than high school graduate or only a high school graduate); and not being married, be it single, partnered, or widowed/divorced/separated. While marital status is often associated with and/or viewed as a modest proxy for sexual behavior, none of the sexual behavior variables—ever having sex, age at sexual debut, or lifetime number of sexual partners—were significantly associated with increased risk of vaccine type HPV. No variables in the GEE model were found to have a statistically significant association with decreased risk of vaccine-type HPV.

3.4 Discussion

Substantial and statistically significant decreases in the prevalence of preventable vaccine-type HPV infections were observed in females under the age of 20 years between the pre- and post-vaccine periods from the NHANES Vaginal Swab Surveys. From the entire sample of females aged 14-59 years, only trends in decreased HR and LR vaccine-type HPV infections were documented. These decreases of vaccine-type HPV—namely HR HPV 16 and LR HPV 6—in the younger females confirm previously published ecological study data indicating decreased vaccine-type HPV prevalence after the advent and widespread use of HPV vaccination in U.S. and Australian females.[44-46] Moreover, the observed decreases in vaccine-type HPV prevalence at the population level validate results generated in the pivotal phase III trials of both the bivalent and quadrivalent vaccines[36,37] as well as the numerous mathematical modeling studies assessing the virological impact of HPV vaccination.[62-65]

In analyzing vaccine-type HPV prevalence between the two periods in the youngest age group, the decrease in vaccine-type prevalence was most pronounced in females 18 and 19 years old. In these older adolescents, combined HR vaccine-type prevalence declined approximately 70%, and almost 80% in LR vaccine-type prevalence.

There were even sizable prevalence decreases in specific individual vaccine-type HPV genotypes in the youngest age group. A 70% and ~50% decrease in prevalence was observed in HPV 6 and HPV 16, respectively. Due to limited observations and a relative standard error of $\geq$30% in this age group, the substantial decrease observed in HPV 18 did not reach statistical significance.

Limited self-reported data are available on the particulars of HPV vaccination status of females in the 2007-2008 and 2009-2010 NHANES HPV DNA Vaginal Swab Surveys. Vaccine information, in the form of two questions—received the vaccine (yes or no) and number of doses—is the extent of the information from females in the Vaginal Swab surveys who answered the NHANES Immunization (IMQ_E and IMQ_F) questionnaire.

As expected, history of vaccination was inversely correlated with age: 34.1%, 15.8%, and 7.4% of females aged 14-19, 20-24, and 25-29 years, respectively. Of those reporting receipt of $\geq$1 dose, 62.5%, 43.2% and 45.7% said they received all 3 recommended doses. The 34.1% rate of receiving $\geq$1 dose documented in the NHANES adolescents is considerably lower than the national average. The physician-verified CDC-sponsored NIS-Teen Survey reported that 48.7% adolescent females received $\geq$1 HPV vaccine dose in 2010.[52]

Nevertheless, and unsurprisingly, the largest difference in vaccine-type HPV prevalence was observed when comparing females who received $\geq$1 vaccine dose to those unvaccinated. While the 58% reduction of vaccine-type HR HPV and 85% decrease in vaccine-type LR HPV in vaccinated females is impressive, only statistical significance was reached in the LR vaccine-type comparison.

Such markedly reduced vaccine-type HPV prevalence in vaccinated versus unvaccinated females has been previously documented in two published ecological studies.[44,45] Our analyses differ from two other ecological studies due to the inclusion of older females up to age 29 years. The 2013 NHANES analysis [44] included females aged 14-19 years, whereas the Australian study sampled females aged 18-24 years. Of note, ~86% of the Australian females had a history of

HPV vaccination compared to ~21% of similar-aged females in the NHANES Vaginal Swab Survey.

No significant difference in weighted socio-demographic variables or reported sexual behavior was observed at baseline between the pre- and post-vaccine period females. In the multivariate analyses, younger age, lower education level, and not being married were significantly associated with increased vaccine-type HPV prevalence, and no risk factors were found to be protective of decreased prevalence. These results differ from our multivariate model, as shown in **Table 3.8c**, of risk factors associated with all 37 HPV genotypes combined. Here, non-white race, ever having sex, and multiple lifetime number of sexual partners were found to be statistically significantly associated with increased risk of all 37 HPV genotypes combined.

In numerous longitudinal studies, younger age—most likely because of its correlation with sexual partnership—has been associated with increased risk of HPV.[47,66,67] Our finding that not being married is associated with increased risk of HPV confirms a number of previously published studies.[68-70] Unlike many other analyses that have documented higher lifetime number of sexual partners as a distinct vaccine-type HPV risk factor,[12,47,67,71-75] this categorized sexual behavior variable did not reach statistical significance in pre- and post-vaccine period females.

There are conflicting data that suggest non-white race is independently associated with LR and/or HR HPV DNA. Our results confirm a previously published analysis which did not find an association between nonwhite race and vaccine-type HPV infection.[66] It does, however, conflict with results from two studies documenting increased risk of HPV infection in non-Hispanic black females.[75,76]

Our analyses do not suggest the possibility that a reduction in vaccine-type HPV genotypic prevalence was occurring in the pre-vaccine era. When comparing vaccine-type HPV prevalence between 2003-2004 and 2005-2006, no statistically significant decrease or increase was observed. This does conflict with the results from a recently published Australian study which observed a trend towards decreased prevalence in the latter years of the pre-vaccine era.[45]

We are uncertain as to why there was only a modest and insignificant difference in vaccine-type HR HPV prevalence between the aggregated top-10 and bottom-10 states in HPV vaccine coverage. While data are available from the 2010 NIS-Teen Survey on vaccine coverage of the top-10 states and the bottom-10 states, the CDC's NCHS does not allow information of exact geographic location (e.g., state) of study participants due to patient confidentiality concerns. The smallest unit of analysis the NCHS allows is the 10-state aggregation with a proviso that participants simply "resided in one of the ten states." Thus, it remains unclear how well distributed the post-vaccine era females were geographically.

Strengths & Limitations

A significant strength of these analyses comes from access to publicly available NHANES data with its many years of well-characterized sampling method protocols and quality-control laboratory manuals. NHANES use of weighting and over-sampling of African Americans, Asians, and Hispanics speaks to its rigorous inclusion of myriad U.S. and foreign-born populations in order to achieve a nationally representative sample.

The HPV DNA Vaginal Swab Survey, like many others in NHANES, is unique in that it combines interviews, physical examinations, and specimen collection. No other population-based survey in the U.S. or internationally has continuously collected HPV DNA genotypic data

along with risk factor information including socio-demographic information, lifestyle, and sexual behavior history.

An additional strength of the analyses is that it is the first reported comparison of individual and grouped vaccine-type HPV DNA genotypic prevalence in a wide age range of females from 14 to 59 years before and after the advent of HPV vaccination in 2006. Likewise, this is the first reported geographic analysis of HPV prevalence to be conducted in the history of the NHANES Vaginal Swab Surveys.

There are also a number of limitations to these analyses. By their nature, the use of population-level data rather than individual-level data in these ecological analyses hampered our ability to assess causation between exposure and outcome. While there are specific data on HPV genotype prevalence from the pre- and post-vaccination periods, no concrete, physician-verified data exist to determine if these females in the latter period were actually vaccinated. It is only self-report data.

My geographical comparator groups—the top-10 and bottom-10 states in vaccine coverage—can be considered crude and somewhat arbitrary. As noted, this was the lowest geographic unit of analysis the CDC's NCHS would make available. Nonetheless, the use of the CDC-sponsored NIS-Teen Survey for quantifying vaccine coverage by state is considered the most accurate of all immunization surveys because of its use of physician verification.[53]

While NHANES strives to conduct its samples with rigor and has excellent laboratory quality control, there are no other evaluations in the general U.S. population using self-collected vaginal swabs that would have allowed for a direct comparison of past or present HPV DNA prevalence. Also, NHANES data only offer HPV DNA point prevalence. Because HPV DNA

genotypes often clear in females in 6-12 months,[77] this point prevalence is certain to underestimate cumulative incidence. Moreover, the HPV DNA vaginal swab sampling only measures current infection and does not indicate past exposure (and thus clearance) to HPV. This, however, is neither the fault of NHANES sampling nor its laboratory assay. No assay currently exists to identify past/cleared HPV infection, the duration of given infection, or if a particular HPV genotype is either new or a reinfection.

Another limitation is the inability to control for confounding of vaccine uptake in males in the two 10-state comparison groups. While Merck did receive an additional indication from the FDA for Gardasil to be administered to males aged 9-26 years in 2009, data from the 2011 NIS-Teen Survey on HPV vaccination coverage in males documents a national average of only 8.3% in boys aged 13-17 years.[78] Of note, only 19 states reported HPV vaccination coverage in males to CDC's 2011 NIS-Teen Survey. With male HPV vaccine coverage extremely low in states and/or not reported, it was not feasible to control for male HPV vaccination.

Public Health Significance

These analyses of temporal trends in vaccine-type HPV prevalence between the pre- and post-vaccine periods at the population level have obvious and important public health consequences. It is, of course, too early to determine if HPV vaccination in females has had an impact in preventing ICC. *Healthy People 2020* states a goal of HPV vaccination is to "reduce the death rate from cancer of the uterine cervix below a target of 2.2 deaths/100,000 females (from a baseline of 2.4 per 100,000 in 2007)."[79] Thus, the monitoring of early vaccine impact, namely a reduction of HPV genotypes 16 and 18 at the population level, is an integral part of the spectrum of intermediate outcomes of HPV vaccination. This investigation lends a voice to the

CDC's assertion that observing early virological outcomes of the vaccines is a "critical aspect of monitoring its population impact."[43]

The confirmation of marked reduction in HPV 16 in adolescent females will be useful on a public policy level in order to advocate for increased HPV vaccination coverage (and education) in the U.S. of both young females and males. Since 2006, HPV vaccine uptake in adolescent females has steadily increased, however, at a much slower rate than expected with a U.S. average of ~53% completion of 3 doses in females aged 13-17 years during 2011.[78] Low level of coverage cannot necessarily be attributed to cost as the CDC-sponsored Vaccine for Child (VFC) Program[3] and Section 317 grants to states cover the expense for low-income families. This knowledge of the vaccine's potent activity at the population level in preventing HPV 16—the most common genotype associated with ICC as well as head and neck cancers—will offer healthcare professionals further proof of the vaccine's effectiveness. Armed with this information, physicians need to help dissuade parents' notion that HPV vaccination is "not necessary or needed."[80] Undoubtedly, greater vaccine uptake in females due to the understanding that the vaccine is doing what it's meant to—preventing a cancer-causing virus—will undoubtedly help normalize and increase HPV vaccine uptake in males to prevent anal, penile, and head and neck cancers.

[3] The VFC program provides vaccines at no cost to doctors who serve eligible children. Children younger than 19 years of age are eligible for VFC vaccines if they are Medicaid-eligible, American Indian or Alaska Native or have no health insurance.

Conclusion

We specifically documented that vaccine-type LR and HR HPV prevalence markedly decreased in females aged 14-19 years who are part of the recommended vaccination age group. The present research adds to the accumulating body of evidence that HPV vaccination in adolescent females has profound virologic activity in reducing vaccine-type HPV genotypic prevalence and the early clinical sequel of HPV infection.[44-46,81-86] Further studies are needed to ascertain if the vaccination provides cross-protection against non-vaccine-targeted HR HPV genotypes, and if there is any evidence of HR HPV type-replacement. Moreover, future ecological studies assessing temporal trends in vaccine-type HPV prevalence will need to include males. If these studies can obtain physician-verified vaccine history of participants as well as male sexual behavior (e.g., heterosexual or homosexual), it may be possible to determine if decreased HPV prevalence is due, in part, to the effect of herd immunity.

Finally, with such strong mounting evidence of early virologic activity and a stellar safety profile, it is time for public health officials to strongly consider mandatory HPV vaccination for all school-aged females. By using the model of Hepatitis B virus (HBV) vaccination programs, this will be the surest way to normalize HPV vaccination and prevent ICC and other HPV-associated cancers at the population level.[87]

3.5 Tables & Figures

Table 3.1. Demographics: weighted distribution of potential confounders in the pre- and post-vaccination periods

Variables	Categorization	2003-2006 (N=4990) %	2007-2010 (N=4860) %
Age	14-19 years	13.4	13.0
	20-24 years	11.1	10.5
	25-29 years	9.8	10.9
	30-39 years	21.6	20.8
	40-49 years	24.5	23.5
	50-59 years	19.7	21.3
Race/ethnicity	Non-Hispanic white	67.7	64.6
	Non-Hispanic black	13.4	13.2
	Mexican American	8.7	9.2
	Other	10.1	13.0
Education[a]	Less than high school	14.3	17.7
	High school graduate	23.3	21.5
	More than high school	62.4	60.7
Marital status	Married	49.6	54.7
	Widowed/divorced/separated	14.3	15.9
	Never married	28.7	20.8
	Living with partner	7.4	8.6
Age at sexual debut	<16 Years	39.4	40.6
	>16 Years	56.5	55.5
	Never had sex	4.2	3.9
Total lifetime sex partners	0	0.4	2.3
	1	18.0	16.3
	2	10.1	10.2
	3-5	28.7	29.4
	≥6	42.8	41.7

Table 3.2. Age distribution of females between the pre- and post-vaccine periods

Age Group	2003-2006 (N=4,990)	2007-2010 (N=4,860)
14-19 years	N=1,660	N=887
20–24 years	N=551	N=513
25–29 years	N=523	N= 472
30–39 years	N=850	N= 1,034
40–49 years	N=795	N= 1,072
50–59 years	N=611	N= 882

Table 3.3a. Vaccine-type HPV prevalence among the entire sample of females (ages 14-59 years)

HPV Type	Prevalence % (95% CI)		Unadjusted Prevalence Ratio[a] (95% CI)	Adjusted[b] Prevalence Ratio[a] (95% CI)
	2003-2006 (N=4990)	2007-2010 (N=4860)		
Any HPV[c]	37.0 (37.0-38.7)	35.0 (33.3-36.6)	0.95 (0.88-1.01)	0.96 (0.89-1.03)
All vaccine type[d]	7.7 (6.7-8.6)	6.8 (6.0-7.6)	0.89 (0.74-1.06)	0.99 (0.81-1.21)
HR vaccine type[e]	5.4 (4.6-6.2)	5.3 (4.6-6.1)	0.99 (0.81-1.22)	Suppressed due to small cells
LR vaccine type[f]	2.8 (2.2-3.3)	1.7 (1.3-2.2)	0.63 (0.46-0.87)	0.81 (0.55-1.19)

[a] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance[57]
[b] Adjusted for age; race/ethnicity; education; marital status; age at sexual debut; and lifetime number of sexual partners
[c] All 37 genotypes: HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39
[d] Vaccine-type HPV combine: HPV 6, 11, 16, and 18
[e] HR vaccine-type: HPV 16 and/or 18
[f] LR vaccine-type: HPV 6 and/or 11
* P<0.05

Table 3.3b. HR HPV vaccine-type prevalence between the 1st & 2nd two-year surveys of the pre-vaccine period

	2003-2004 Prevalence % n= 2387	2004-2005 Prevalence % n= 2603	P-Value
HR HPV vaccine-type[a]	4.7	6.0	NS

[a] HR vaccine type: HPV 16 and/or 18
[b] Exponentiated prevalence ratio calculated using Zou's "modified Poisson" regression model with a robust error variance[57]

Table 3.4. Vaccine-type HPV prevalence in females stratified by age group

Age	HPV Type	2003-2006 (% & 95% CI)	2007-2010 (% & 95% CI)	Prevalence Ratio[a] (95% CI)
14-19 y/o		*n= 1660*	*n= 887*	
	LR vaccine-type[b]*	8.1 (5.0-11.3)	2.2 (0.79-3.6)	0.27 (0.13-0.56)
	HR vaccine-type[c]*	6.0 (4.5-7.5)	2.9 (1.9-4.0)	0.49 (0.32-0.76)
20-24 y/o		*n=551*	*n=558*	
	LR vaccine-type	3.5 (1.6-5.4)	4.3 (2.3-6.3)	1.23 (0.60-2.51)
	HR vaccine-type	12.7 (9.0-16.3)	13.8 (10.0-17.5)	1.09 (0.73-1.61)
25-29 y/o		*n=523*	*n=540*	
	LR vaccine-type	3.4 (1.5-5.3)	3.1 (1.3-5.0)	0.92 (0.41-2.06)
	HR vaccine-type	6.9 (4.1-9.8)	9.1 (6.0-12.2)	1.32 (0.77-2.25)
30-39 y/o		*n=850*	*n=1,062*	
	LR vaccine-type	2.8 (1.4-4.2)	1.3 (0.54-2.0)	0.45 (0.21-0.97)
	HR vaccine-type	5.9 (3.9-7.8)	5.0 (3.6-6.4)	0.85 (0.55-1.31)
40-49 y/o		*n=795*	*n=1,164*	
	LR vaccine-type	1.6 (0.54-2.6)	1.2 (0.52-2.0)	0.78 (0.32-1.87)
	HR vaccine-type	3.0 (1.5-4.5)	4.0 (2.6-5.5)	1.35 (0.73-2.48)
50-59 y/o		*n=611*	*n=888*	
	LR vaccine-type	1.6 (0.59-2.7)	1.0 (0.21-1.8)	0.61 (0.22-1.67)
	HR vaccine-type	2.6 (1.2-4.0)	2.5 (1.3-3.8)	0.98 (0.48-1.99)

[a] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance[57]

[b] LR vaccine type: HPV 6 and/or 11

[c] HR vaccine type: HPV 16 and/or 18

* P<0.05

Table 3.5a. Weighted prevalence of individual vaccine-type HPV genotypes among the entire sample from the NHANES HPV Vaginal Swab Surveys, 2003-2006 and 2007-2010

	2003-2006 (n=4,990)			2007-2010 (n=4,860)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6	153	2.8	(2.2-3.4)	95	1.8	(1.4-2.3)
11	20	0.3	(0.1-0.5)	Suppressed: RSE ≥30% or <10 observations		
16	202	4.6	(3.8-5.4)	203	4.6	(3.9-5.4)
18	88	1.8	(1.3-2.3)	92	1.6	(1.2-2.0)

RSE: Relative Standard Error

Table 3.5b. Females aged 14-19 years

	2003-2006 (n=1,660)			2007-2010 (n=887)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6*	79	5.3	(3.7-6.8)	20	1.6	(0.9-2.4)
11	14	1.0	(0.3-1.7)	Suppressed: RSE ≥30% or <10 observations		
16*	82	5.8	(4.2-7.5)	31	3.0	(1.8-4.1)
18	33	1.6	(0.9-2.3)	10	1.0	(0.2-1.7)

*p<.05; RSE: Relative Standard Error

Table 3.5c. Females aged 20-29 years

	2003-2006 (n=1,074)			2007-2010 (n=985)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6	33	3.8	(2.9-5.3)	28	2.9	(1.7-4.0)
11	1	0.2	(0.0-0.6)	8	0.5	(0.2-0.9)
16	67	9.6	(7.1-12.2)	94	10.2	(7.9-12.4)
18	20	2.7	(1.4-4.0)	32	3.3	(2.0-4.6)

Table 3.5d. Females aged 30-39 years

	2003-2006 (N=850)			2007-2010 (N=1,034)		
HPV Type	**Number Positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6	18	3.0	(1.5-4.5)	18	1.7	(0.8-2.6)
11	2	0.16	(0.4-0.7)	Suppressed: RSE of ≥30% or <10 observations		
16	25	4.2	(2.3-6.0)	40	3.9	(2.5-5.3)
18	21	2.8	(1.4-4.2)	24	1.9	(1.0-2.9)

RSE: Relative Standard Error

Table 3.5e. Females aged 40-49 years

	2003-2006 (n=795)			2007-2010 (n=1,072)		
HPV Type	**Number Positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6	12	1.4	(0.4-2.4)	16	1.0	(0.4-1.7)
11	2	0.4	(0.0-1.0)	Suppressed: RSE of ≥30% or <10 observations		
16	13	2.2	(0.8-3.5)	24	2.6	(1.4-3.9)
18	8	1.2	(0.1-2.2)	26	1.6	(0.9-2.2)

RSE: Relative Standard Error

Table 3.5f. Females aged 50-59 years

	2003-2006 (n=611)			2007-2010 (n=882)		
HPV Type	**Number Positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
6	11	1.6	(0.5-2.8)	12	1.1	(0.2-2.0)
11	1	0.2	(0.0-0.5)	4	0.2	(0.0-0.4)
16	15	2.2	(0.8-3.5)	14	1.3	(0.4-2.3)
18	6	0.8	(0.1-1.4)	18	2.1	(0.9-3.3)

Table 3.6. Weighted vaccine-type HPV prevalence according to vaccination status among females ages 14-29 years from the post-vaccine period (2007-2010)

HPV Type	Prevalence (%)		PR (95% CI)[b]
	Unvaccinated N=1,423	Vaccinated[a] N=412	
Any HPV (of 37 types)	33.9	37.2	1.10 (0.90-1.30)
HR vaccine-type (HPV 16 and/or 18)	9.3	3.9[ŧ]	0.42 (0-1.18)
LR vaccine-type (HPV 6 and/or 11)	3.5	0.5[ŧ]	0.15 (0-0.51)*

Abbreviations: CI, confidence interval; PR, prevalence ratio; HR, high risk; LR, low risk; RSE, relative standard error.

[a] Vaccination defined as a self-reported history of receiving ≥1 vaccine dose

[b] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance, *Am J Epidemiol* 2004;159:702–6.

***** $p<0.05$

[ŧ] RSE ≥30%

Table 3.7a. Weighted HR vaccine-type HPV prevalence in 2003-2006 among females who resided in one of the top-10 states with the highest HPV vaccine coverage compared to those who resided in one of the bottom-10 states with the lowest HPV vaccine coverage

HPV Type	Top-10 states in vaccine coverage[b] (combined) 2003-2006	Bottom-10 states in vaccine coverage[c] (combined) 2003-2006	P-value
HR vaccine-type (HPV 16 and/or 18)	5.7%	6.4%	.6836

[a] According to 2010 NIS-Teen published data
[b] Rhode Island; South Dakota; Massachusetts; Connecticut; Washington; Wisconsin; Nebraska; New Hampshire; Pennsylvania; Virginia
[c] Idaho; Arkansas; Mississippi; Alabama; Utah; Georgia; Indiana; Florida; Alaska; Kansas

Table 3.7b. Weighted HR vaccine-type HPV prevalence in 2007-2010 among females who resided in one of the top-10 states with the highest HPV vaccine coverage compared to those who resided in one of the bottom-10 states with the lowest HPV vaccine coverage

HPV Type	Top-10 states in vaccine coverage (combined) 2007-2010	Bottom-10 states in vaccine coverage (combined) 2007-2010	P-value
HR vaccine-type (HPV 16 and/or 18)	4.5%	5.9%	.3804

Table 3.8a. Multivariate GEE analysis of factors associated with LR and HR vaccine-type HPV infection among females aged 20-59 years from the pre- and post-vaccine periods

Variables	Categorization	Risk Ratio & (95% CI)	P-value
Age	20-24 years	2.80 (1.90-4.14)	<0.0001
	25-29 years	2.01 (1.42-3.13)	<0.001
	30-39 years	1.56 (1.06-2.29)	0.02
	40-49 years	1.21 (0.81-1.80)	0.36
	50-59 years	Referent	
Race/ethnicity	Non-Hispanic white	Referent	
	Non-Hispanic black	1.03 (0.83-1.30)	0.79
	Mexican American	0.84 (0.63-1.12)	0.23
	Other	0.80 (0.57-1.12)	0.19
Education	Less than high school	1.48 (1.14-1.91)	<0.01
	High school graduate	1.71 (1.35—2.16)	<0.0001
	More than high school	Referent	
Marital status	Married	Referent	
	Widowed/divorced/separated	1.43 (1.04-1.97)	0.03
	Never married	2.28 (1.71-3.03)	<0.0001
	Living with partner	2.13 (1.51-3.0)	<0.0001
Age at sexual debut	Never had sex	Referent	
	<16 Years	5.46 (0.54-54.87)	0.15
	>16 Years	5.68 (0.57-56.96)	0.14
Total lifetime sex partners	0	Referent	
	1	0.69 (0.19-2.46)	0.57
	2	1.42 (0.41-4.90)	0.57
	3-5	1.82 (0.55-5.98)	0.32
	≥6	2.76 (0.84-8.98)	0.09

Table 3.8b. Multivariate GEE analysis of factors associated with any HPV infection among females aged 20-59 years from the pre- and post-vaccine periods

Variables	Categorization	Risk Ratio & (95% CI)	P-value
Age	20-24 years	1.32 (1.16-1.49)	<.0001
	25-29 years	1.16 (1.02-1.32)	0.02
	30-39 years	1.08 (0.96-1.21)	0.12
	40-49 years	1.03 (0.92-1.16)	0.57
	50-59 years	Referent	
Race/ethnicity	Non-Hispanic white	Referent	
	Non-Hispanic black	1.24 (1.15-1.33)	<.0001
	Mexican American	1.26 (1.15-1.37)	<.0001
	Other	1.16 (1.03-1.30)	0.01
Education[a]	Less than high school	1.18 (1.09-1.29)	<.0001
	High school graduate	1.24 (1.14-1.35)	<.0001
	More than high school	Referent	
Marital status	Married	Referent	
	Widowed/divorced/separated	1.56 (1.42-1.71)	<.0001
	Never married	1.43 (1.29-1.57)	<.0001
	Living with partner	1.46 (1.30-1.64)	<.0001
Age at sexual debut	Never had sex	Referent	
	<16 Years	3.10 (1.40-6.87)	<0.01
	>16 Years	3.22 (1.45-7.11)	<0.01
Total lifetime sex partners	0	Referent	
	1	0.55 (0.33-0.91)	0.02
	2	0.97 (0.59-1.60)	0.91
	3-5	1.39 (0.86-2.23)	0.19
	≥6	1.79 (1.12-2.88)	0.02

Figure 3.1a. Top-10 states with the highest estimated HPV vaccination coverage (all 3 doses) - NIS-Teen Survey, 2010

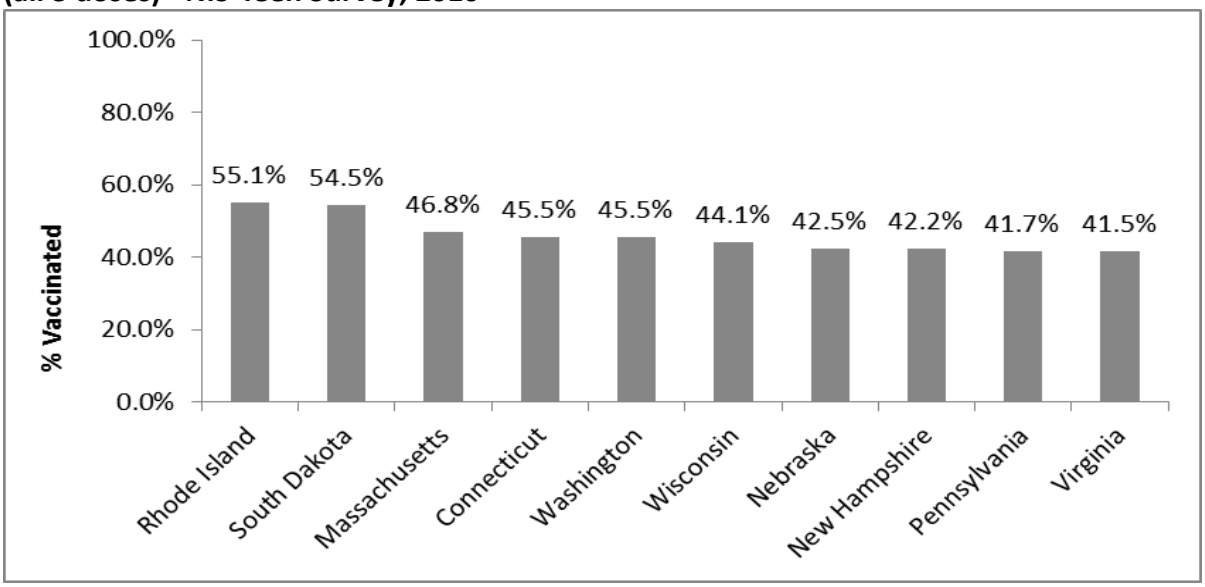


Figure 3.1b. Bottom-10 States with the lowest estimated HPV vaccination coverage (all 3 doses) - NIS-Teen Survey, 2010

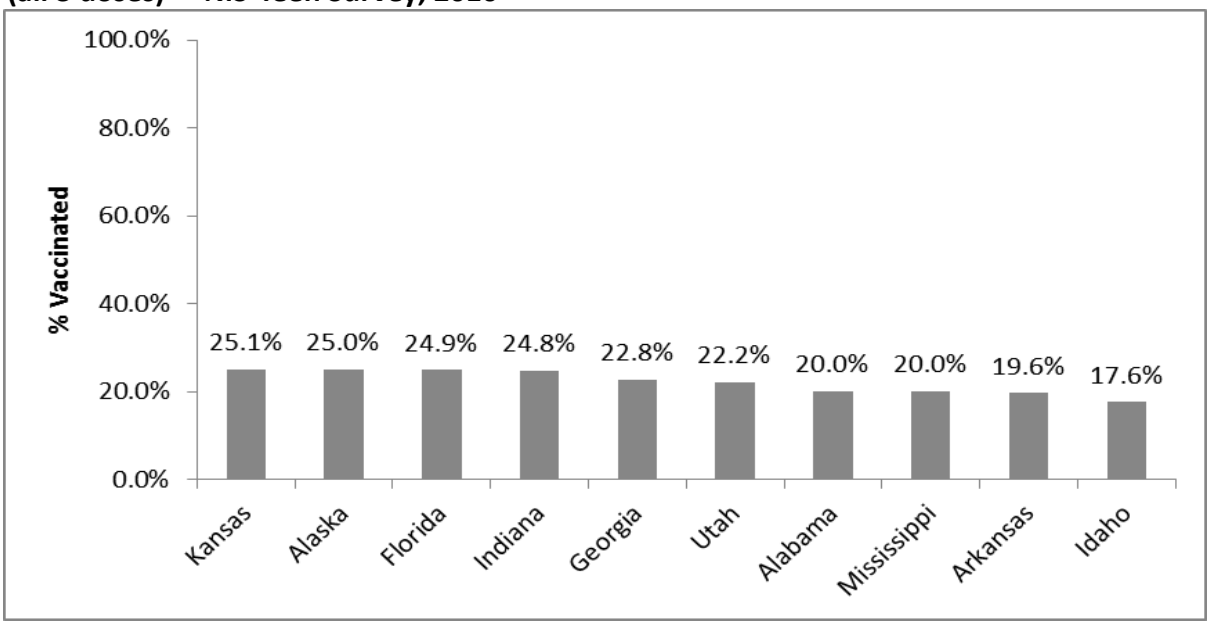
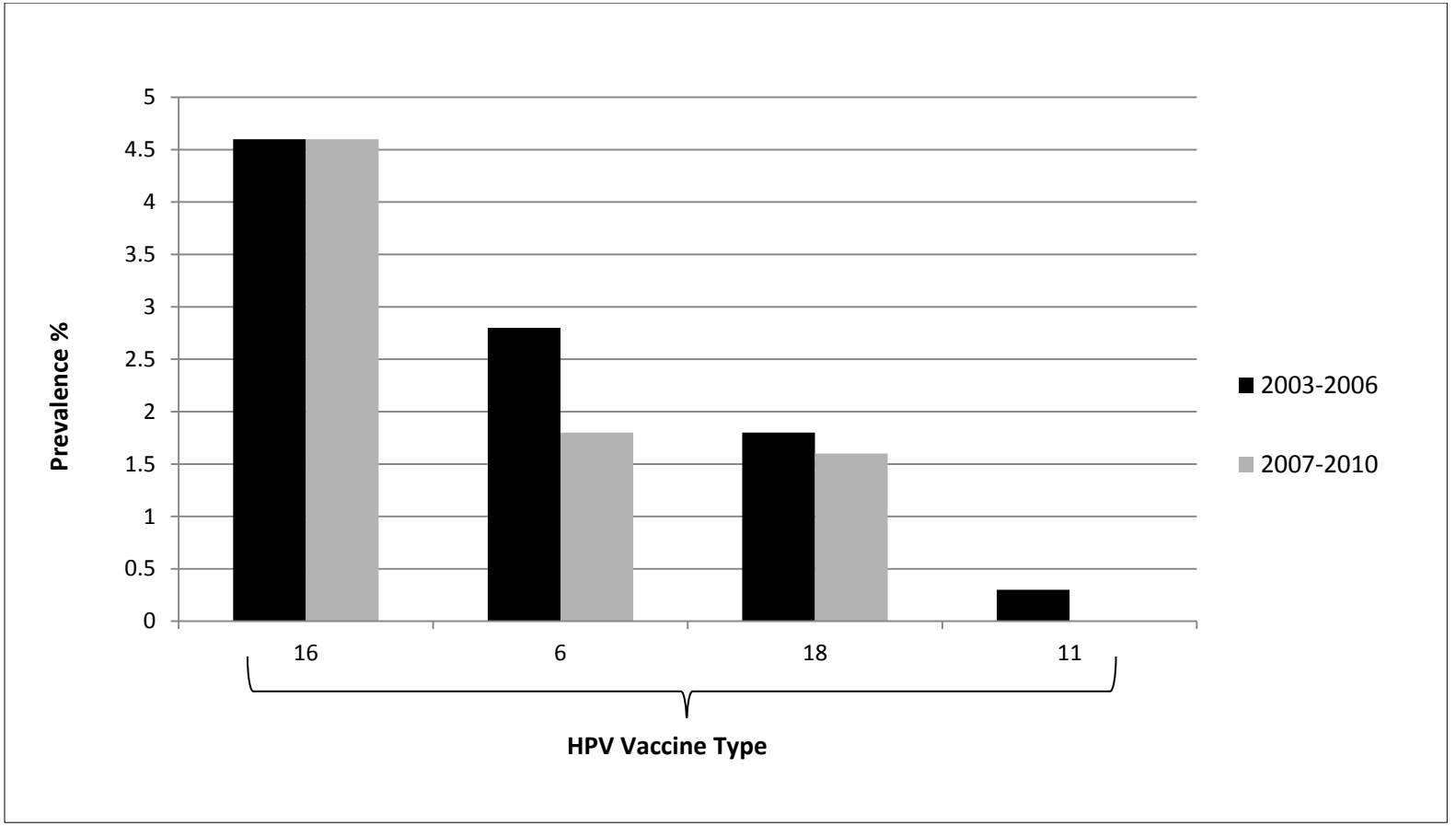
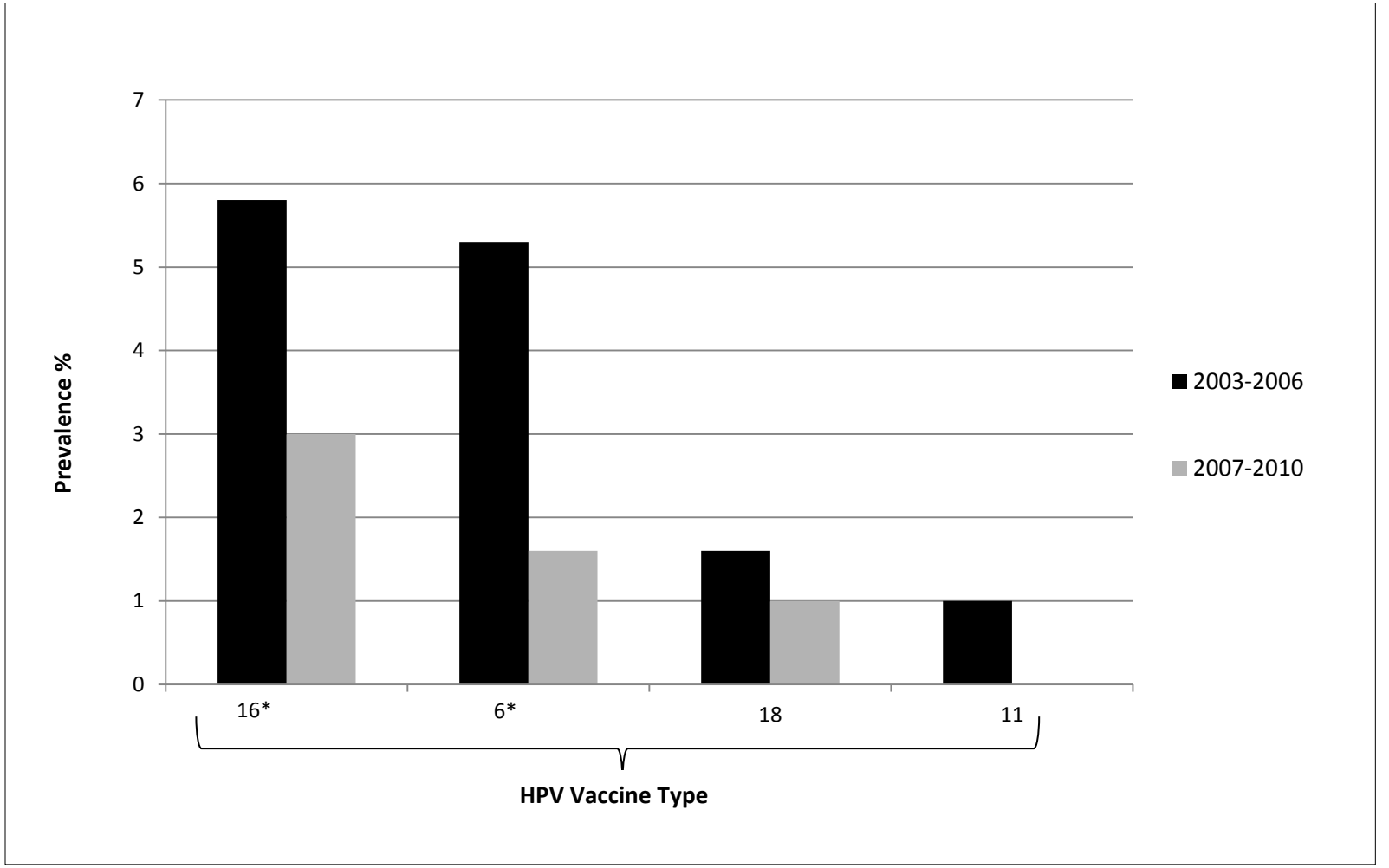


Figure 3.2a. Vaccine-type HPV genotypic prevalence in all females between pre- and post-vaccine periods



Missing bars in 2007-2010 denote a relative standard error of ≥30% or <10 outcomes

Figure 3.2b. Vaccine-type HPV genotypic prevalence in females 14-19 y/o between pre- and post-vaccine periods



*P<0.05; Missing bars in 2007-2010 denote a relative standard error of ≥30% or <10 outcomes

3.6 References

1. Hebner CM, Laimins LA. Human papillomaviruses: Basic mechanisms of pathogenesis and oncogenicity. Rev Med Virol 2006;16:83-97.

2. Stanley M. Immune responses to human papillomavirus. Vaccine 2006;24:S16-S22.

3. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: human papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007:1-636.

4. Munoz N, Bosch FX, De Sanjose S, et al. The causal link between human papillomavirus and invasive cervical cancer: A population-based case-control study in Colombia and Spain. Int J Cancer 1992;52:743-9.

5. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.

6. Hoots, BE, Palefsky, JM, Pimenta, JM, Smith, JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int J Cancer 2009; 124:2375-2383.

7. Franceschi S, Muñoz N, Bosch XF, Snijders PJF, Walboomers JMM. Human papillomavirus and cancers of the upper aerodigestive tract: A review of epidemiological and experimental evidence. Cancer Epidemiol Biomarkers Prev 1996;5:567-75.

8. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 1989;115:621-5.

9. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709-20.

10. Frisch M, Fenger C, Van Den Brule AJC, et al. Variants of squamous cell carcinoma of the anal canal and perianal skin and their relation to human papillomaviruses. Cancer Research 1999;59:753-7.

11. Weinstock H, Berman S, Cates Jr W. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Repro H 2004;36:6-10.

12. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-8.

13. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218-26.

14. Franceschi S, Herrero R, Clifford GM, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer 2006;119:2677-84.

15. Markowitz LE, Sternberg M, Dunne EF, McQuillan G, Unger ER. Seroprevalence of human papillomavirus types 6, 11, 16, and 18 in the United States: National Health and Nutrition Examination Survey 2003-2004. J Infect Dis 2009;200:1059-67.

16. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. (Accessed November 4, 2013, at http://globocan.iarc.fr.)

17. Franceschi S, Denny L, Irwin KL, et al. Eurogin 2010 roadmap on cervical cancer prevention. Int J Cancer 2011;128:2765-74.

18. Muñoz N, Bosch FX, De Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.

19. Cogliano V, Baan R, Straif K, et al. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6:204.

20. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.

21. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: A meta-analysis. Br J Cancer 2003;89:101-5.

22. de Sanjose S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.

23. Clifford GM, Smith JS, Plummer M, Muñoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: A meta-analysis. Br J Cancer 2003;88:63-9.

24. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76-84.

25. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001;286:3106-14.

26. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol 1969;105:386-93.

27. Hawes SE, Kiviat NB. Screening for cervical cancer. In: Holmes KK, Sparling FP, Stamm WE, et al., eds. Sexually Transmitted Diseases. New York: McGraw-Hill Professional; 2007:1075-104.

28. Jacobs MV, De Roda Husman AM, Van den Brule AJC, et al. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J Clin Microbiol 1995;33:901-5.

29. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol 1995;33:2058-63.

30. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191:731-8.

31. Pirotta M, Ung L, Stein A, et al. The psychosocial burden of human papillomavirus related disease and screening interventions. Sex Transm Infect 2009;85:508-13.

32. Hu D, Goldie S. The economic burden of noncervical human papillomavirus disease in the United States. Am J Obstet Gynecol 2008;198:500.e1-.e7.

33. Markowitz LE, Dunne EF, Saraiya M, et al. Human papillomavirus vaccination recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2014;63:1-30.

34. Petrosky E, Bocchini JA, Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: Updated hpv vaccination recommendations of the advisory committee on immunization practices. MMWR 2015;64:300-4.

35. CDC. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the advisory committee on immunization practices (ACIP). MMWR 2010;59:630-2.

36. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.

37. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374:301-14.

38. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med 2011;364:401-11.

39. Joura EA, Giuliano AR, Iversen O-E, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med 2015;372:711-23.

40. Romanowski B, de Borba PC, Naud P. Sustained efficacy and immunogenicity of the human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine: analysis of a randomised placebo-controlled trial up to 6.4 years. Lancet 2009;374:1975-85.

41. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012;13:89-99.

42. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2008. 2011. National Cancer Institute. Bethesda, MD, 2011. (Accessed June 1, 2014, at http://seer.cancer.gov/csr/1975_2008/.)

43. Hariri S, Markowitz L. Monitoring HPV vaccine impact: Early results and ongoing challenges. J Infect Dis 2012;206:1633-5.

44. Markowitz LE, Hariri S, Lin C, et al. Reduction in Human Papillomavirus (HPV) Prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013; 208:385-393.

45. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645-51.

46. Kahn JA, Brown DR, Ding L, et al. Vaccine-type human papillomavirus and evidence of herd protection after vaccine introduction. Pediatrics 2012;130: e249-e256.

47. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813-9.

48. DHHS. Third National Health and Nutrition Examination Survey, 1988-1994, NHANES III Human papillomavirus-16 antibody data file. In: Prevention CDC, ed. Hyattsville, MD, 2001.

49. CDC. National Center for Health Statistics. NHANES 2003-2004. (Accessed June 2, 2012, at http://www.cdc.gov/nchs/about/major /nhanes/nhanes.)

50. Hariri S, Unger ER, Sternberg M, et al. Prevalence of genital human papillomavirus among females in the United States, the National Health and Nutrition Examination Survey, 2003-2006. J Infect Dis 2011;204:566-73.

51. Van Doorn LJ, Quint W, Kleter B, et al. Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF 10 line probe assay. J Clin Microbiol 2002;40:979-83.

52. Dorell C, Stokley S, Yankey D, Liang JL, Markowitz L. National and state vaccination coverage among adolescents aged 13 through 17 years-United States, 2010. MMWR 2011;60:1117-23.

53. Tiro JA, Saraiya M, Jain N, et al. Human papillomavirus and cervical cancer behavioral surveillance in the US. Cancer 2008;113:3013-30.

54. NCHS. Analytic note regarding 2007-2010 survey design changes and combining data across other survey cycles. 2011. (Accessed February 7, 2013, at http://www.cdc.gov/nchs/data/nhanes/analyticnote_2007-2010.pdf.)

55. NCHS. National Health and Nutrition Examination Survey, 2011–2012 [Overview]. . 2013. (Accessed March 14, 2013, at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/2011-12_overview_brochure.pdf.)

56. Korn EL, Graubard BI. Confidence intervals for proportions with small expected number of positive counts estimated from survey data. Surv Methodol 1998;24:193-201.

57. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702-6.

58. Fang J. Using SAS Procedures FREQ, GENMOD, LOGISTIC, and PHREG to estimate adjusted relative risks – a case study. SAS Global Forum 2011, 4–11 April 2011. Las Vegas: SAS Institute Inc.; 2011, 345-2011.

59. Satterthwaite FE. Synthesis of variance. Psychometrika 1941;6:309-16.

60. Freund RJ, Littell RC. SAS System for Regression. 1986 ed. Cary, NC: SAS Institute Inc.; 1986.

61. SAS Institute Inc. SAS® 9.4 Guide to Software Updates. Cary, NC: SAS Institute Inc.; 2013.

62. Bogaards JA, Coupe VMH, Xiridou M, et al. Long-term impact of human papillomavirus vaccination on infection rates, cervical abnormalities, and cancer incidence. Epidemiology 2011;22:505-15.

63. Smith MA, Canfell K, Brotherton JM, Lew JB, Barnabas RV. The predicted impact of vaccination on human papillomavirus infections in Australia. Int J Cancer 2008;123:1854-63.

64. Drolet M, Boily MC, Van de Velde N, Franco EL, Brisson M. Vaccinating girls and boys with different human papillomavirus vaccines: Can it optimise population-level effectiveness? PloS One 2013;8:e67072.

65. Malagón T, Joumier V, Boily MC, et al. The impact of differential uptake of HPV vaccine by sexual risks on health inequalities: A model-based analysis. Vaccine 2013;31:1740-7.

66. Wiley DJ, Masongsong EV, Lu S, et al. Behavioral and sociodemographic risk factors for serological and DNA evidence of HPV6, 11, 16, 18 infections. Cancer Epidemiol 2012;36:e183-e9.

67. Bauer HM, Hildesheim A, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. Sex Transm Dis 1993;20:274-8.

68. Kahn JA, Lan D, Kahn RS. Sociodemographic factors associated with high-risk human papillomavirus infection. Obstet Gynecol 2007;110:87-95.

69. Kreimer AR, Struyf F, Del Rosario-Raymundo MR, et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: Combined analysis of data from the Costa Rica Vaccine and PATRICIA trials. Lancet Oncol 2015;16:775-86.

70. Giuliano AR, Papenfuss M, Schneider A, Nour M, Hatch K. Risk factors for high-risk type human papillomavirus infection among Mexican-American women. Cancer Epidemiol Biomarkers Prev 1999;8:615-20.

71. Wheeler CM, Parmenter CA, Hunt WC, et al. Determinants of genital human papillomavirus infection among cytologically normal women attending the University of New Mexico student health center. Sex Transm Dis 1993;20:286-9.

72. Dempsey AF, Gebremariam A, Koutsky LA, Manhart L. Using risk factors to predict human papillomavirus infection: implications for targeted vaccination strategies in young adult women. Vaccine 2008;26:1111-7.

73. Ferrera A, Velema JP, Figueroa M, et al. Co-factors related to the causal relationship between human papillomavirus and invasive cervical cancer in Honduras. Int J Epidemiol 2000;29:817-25.

74. Tarkowski TA, Koumans EH, Sawyer M, et al. Epidemiology of human papillomavirus infection and abnormal cytologic test results in an urban adolescent population. J Infect Dis 2004;189:46-50.

75. Stone KM, Karem KL, Sternberg MR, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396-402.

76. Hariri S, Dunne EF, Sternberg M, et al. Seroepidemiology of human papillomavirus type 11 in the United States: Results from the third national health and nutrition examination survey, 1991-1994. Sex Transm Dis 2008;35:298-303.

77. Giuliano AR, Harris R, Sedjo RL, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 2002;186:462-9.

78. Dorell C, Stokley S, Yankey D, et al. National and state vaccination coverage among adolescents aged 13-17 years - United States, 2011. MMWR 2012;61:671-7.

79. DHHS. Healthy people 2020: immunization and infectious disease. (Accessed April 4, 2013, at http://www.healthypeople.gov/2020/topicsobjectives2020/pdfs/Immunization.pdf.)

80. Garcini LM, Galvan T, Barnack-Tavlaris JL. The study of human papillomavirus (HPV) vaccine uptake from a parental perspective: a systematic review of observational studies in the United States. Vaccine 2012;30:4588-95.

81. Brotherton JML, Fridman M, May CL, et al. Early effect of the HPV vaccination programme on cervical abnormalities in Victoria, Australia: An ecological study. Lancet 2011;377:2058-92.

82. Read TRH, Hocking JS, Chen MY, et al. The near disappearance of genital warts in young women 4 years after commencing a national human papillomavirus (HPV) vaccination programme. Sex Transm Infect 2011;87:544-7.

83. Leval A, Herweijer E, Arnheim-Dahlstrom L, et al. Incidence of genital warts in Sweden before and after quadrivalent human papillomavirus vaccine availability. J Infect Dis 2012;206:860-6.

84. Baandrup L, Blomberg M, Dehlendorff C, et al. Significant decrease in the incidence of genital warts in young Danish women after implementation of a national human papillomavirus vaccination program. Sex Transm Dis 2013;40:130-5.

85. Bauer HM, Wright G, Chow J. Evidence of human papillomavirus vaccine effectiveness in reducing genital warts: An analysis of California public family planning administrative claims data, 2007-2010. Am J Public Health 2012;102:833-5.

86. Powell SE, Hariri S, Steinau M, et al. Impact of human papillomavirus (HPV) vaccination on HPV 16/18-related prevalence in precancerous cervical lesions. Vaccine 2012;31:109-13.

87. Bosch FX, Tsu V, Vorsters A, Van Damme P, Kane MA. Reframing cervical cancer prevention. Expanding the field towards prevention of human papillomavirus infections and related diseases. Vaccine 2012;30 Suppl 5:F1-11.

4.0 Chapter 4:

An investigation of HPV vaccine genotypic cross-protection and type-replacement

Abstract

The aim of the present ecological study is to explore for evidence of HPV genotypic cross-protection and type-replacement at the population level in the first four years after the 2006 commercialization of the first-generation quadrivalent HPV vaccine, Gardasil. It is necessary to determine if vaccinating against high-risk (HR) HPV 16 and 18, the two genotypes responsible for approximately 70% of invasive cervical cancer (ICC), confers additional virologic activity, such as the beneficial effect of preventing non-vaccine-targeted HR HPV genotypes (cross-protection), or the deleterious effect of increasing prevalence of one or more phylogenetically related HR HPV genotypes (type-replacement). Data were drawn from more than 8,000 females aged 14-59 years enrolled between 2003-2010 in the NHANES HPV Vaginal Swab Surveys, a population-based, cross-sectional survey collecting HPV DNA specimens as well as socio-demographic and sexual behavior information. Non-vaccine-targeted HR HPV genotypic prevalence was compared between females from the first two 2-year surveys (2003-2004 and 2005-2006; the "pre-vaccine period") and females from the latter two 2-year surveys (2007-2008 and 2009-2010; the "post-vaccine period") with specific analyses that stratified by age groups, states aggregated by highest and lowest vaccine coverage, and prior vaccination history. "Modified Poisson" regression models with adjusted prevalence ratios were used to compare vaccine-type prevalence between vaccine periods, and GEE regression models were employed to determine risk factors associated with non-vaccine-targeted HR HPV infection. Prevalence of non-vaccine-targeted HR HPV decreased between the two periods from 19.8% (95% CI: 17.3-22.3) to 15.6% (95% CI: 12.6-18.5) with a PR=0.79 (95% CI: 0.63-0.99) in females aged 14-19 years irrespective of vaccination status. In vaccinated females compared to those unvaccinated, increased HPV genotypic prevalence was observed for combined non-vaccine-targeted HR HPV

and genotypes from the alpha-7 species, PR=1.27 and 1.58, respectively. No difference in non-vaccine-targeted HR HPV prevalence was found between aggregated states with high versus low vaccine coverage. These findings of decreased non-vaccine-targeted HR HPV, signifying cross-protection, and increased genotypic prevalence in vaccinated females, suggesting type-replacement, warrant confirmation though further study in females vaccinated with the first-generation quadrivalent Gardasil vaccine.

4.1. Introduction

Virologic aspects of HPV

Human papillomavirus (HPV) is a small double-stranded DNA virus which infects cutaneous and mucosal epithelial cells.[1] HPV-infected epithelial cells undergo terminal differentiation encoding eight open reading frames (ORFs) which are transcribed as polycystic mRNAs from a single DNA strand in order to override any normal regulation of differentiation to produce progeny virions. HPV is tissue-tropic and its replication depends on squamous epithelial differentiation.[2]

Of the approximately 120 HPV types that have been identified, 40 types sort into 15 alpha species which infect the genital tract.[3] These 40 HPV types are divided into two groups: high-risk (HR), also referred to as oncogenic or carcinogenic, and low-risk (LR). HR HPV genotypes have been etiologically linked to invasive cervical cancer (ICC)[4,5] as well as vaginal, vulvar, penile, anal, and a subset of head and neck cancers.[6-10] LR HPV 6 and 11 are associated with 90% of penile, vaginal, and anal warts.[11] Genital warts (GWs) are considered the most immediate clinical manifestation of incident HPV infection.[12]

The WHO's International Agency for Research (IARC) has periodically classified and categorized 20 HPV genotypes in varying degrees of high-risk based on their potential carcinogenicity.[13-15] Twelve genotypes, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59, are in Group 1 because of "sufficient evidence for cervical cancer;" HPV 68 is in Group 2A because it is "probably carcinogenic;" and the remaining seven genotypes, HPV 26, 53, 66, 67, 70, 73, 82, are in Group 2B and considered "possibly carcinogenic." [15]

These 20 HR genotypes taxonomically fall into 5 different alpha species: α5, α6, α7, α9 and α11.[16] HPV 16 and 18 are the HR genotypes most strongly associated with approximately 70% of ICC,[17,18] and belong to α9 and α7, respectively. Five other HR HPV genotypes, HPV 31, 33, 35, 52, and 58, exist in the α9 species, while four HR HPV genotypes, 39, 45, 59, and 68, belong to the α7 species.[16] Of these nine HR HPV genotypes from the two species, strong evidence specifically points to HPV 31, 33, 45, 52 as the HR HPV genotypes—besides HPV 16 and 18—which are etiologically linked to ICC.[17,18]

Co-infection with multiple HR HPV types

Co-infection with multiple HR HPV types (also referred to as clustering) from the α7 and α9 species is considered quite common in sexually active females.[19,20] Many longitudinal studies have documented a large proportion of HPV infections are sustained by multiple genotypes.[19-22] In fact, having HPV 16 or 18 (or both) can itself be a risk factor for the development of being infected with other phylogenetically related HR HPV from the α7 and α9 species. For example, it has been documented that females with incident HPV 16 or 18 infection have 5-7 times higher odds of acquiring a subsequent HPV 58 infection than those not infected with either HPV16 or 18.[21]

The true clinical significance of clustering remains unknown. Strong evidence of the deleterious effect of clustering was documented in a 2009 longitudinal study of ~700 females. The females who were co-infected with HPV clusters of types 31-35-56 or 16-51-52 had greater risk of developing CIN 2+.[20]

Clinical sequel of HR HPV infection

Approximately 40% of women who repeatedly test HPV positive (referred to as "persistent") for HPV 16 will go on to develop high-grade (precancerous) cervical intraepithelial neoplasia (CIN).[23] Moreover, there is a twelvefold increase in the risk of developing high-grade CIN in females with persistent HPV 16 and/or 18 infection compared to other HR HPV genotypes.[24] Stage-3 CIN left untreated for many years can invade the base membrane of the epithelium causing ICC.[25,26]

ICC is the fourth most common cancer in U.S. females, with approximately 530,000 cases diagnosed yearly.[27] It is, however, the number one cancer in sub-Saharan African and Southeast Asian females because high quality screening and early and effective treatment are sparse to non-existent.[28] The World Health Organization (WHO) estimates that ICC is responsible for approximately 270,000 deaths worldwide, mainly in developing countries.[27]

Vaccination against HPV

HPV prophylactic vaccination against HPV 6, 11, 16, and 18 has existed in the U.S. since 2006 beginning with the quadrivalent vaccine, Gardasil. Routine HPV vaccination is recommended for all individuals aged 11 or 12 years, and catch-up vaccination for females aged 13 through 26 years and males aged 13 through 21 years.[29-31] There are now three Food and

Drug Administration (FDA)-approved, commercially available HPV vaccines. While they all differ in the number of specific HPV genotypes they prevent, all target HPV 16 and 18. Pivotal studies of the three vaccines reported 90-98% vaccine efficacy on all endpoints (e.g., CIN 2/3 or GWs) and all documented an excellent safety profile.[32,33 34,35]

In December 2014, Gardasil-9 became the most recent HPV vaccine approved by the FDA for use in females and males. It is a nonavalent vaccine, effective against nine HPV genotypes—the four from the original Gardasil, plus another five HR genotypes: HPV 31, 33, 45, 52, and 58.[36] The pivotal study compared the original Gardasil to Gardasil-9 in 14,000 females aged 16 to 26 years that were followed up to 54 months. Non-inferiority was achieved for HPV 6, 11, 16, and 18, and efficacy against persistent HPV infection and/or CIN 2+ caused by HPV 31, 33, 45, 52, and 58 was ~96%.[35]

HPV vaccination and genotypic cross-protection

Cross-protection of five non-vaccine HR HPV genotypes in the α9 species (HPV 31, 33, 35, 52, and 58) was observed in both the Cervarix and original Gardasil HPV vaccine phase III pivotal trials.[33,37-40] Because Cervarix is formulated with an adjuvant, giving it high levels of cross-neutralizing antibodies, cross-protection of non-vaccine-targeted HR HPV genotypes was expected. In analyzing data submitted by GlaxoSmithKline from its new drug application (NDA) for Cervarix, the FDA noted that in the pivotal study, PATRICIA, vaccine efficacy against CIN2+ due to a combination of 12 non-vaccine-targeted HR HPV genotypes (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was 37.4%. Most importantly, efficacy against HPV 31-related CIN2+ was documented to be 89.4%.[38]

In the original Gardasil phase III pivotal trials (FUTURE I and FUTURE II), cross-protection was detected when combining all five non-vaccine-targeted HR HPV genotypes of the α9 species (vaccine efficacy = 35.4%).[37] As with Cervarix, a statistically significant reduction in HPV 31 was observed.

HPV vaccination and the prospect of future HPV-type replacement

The concern that a new ecological niche could be created by a reduction in the prevalence of HR HPV genotypes targeted by the vaccines (HPV 16 and 18) is not without historic precedent. In the late 1990s and early 2000s, there was documentation of significant increases in the prevalence of non-vaccine serotypes that occurred after the wide-spread introduction of a heptavalent conjugate pneumococcal vaccine and a Bordetella pertussis vaccine.[41-45]

While it is an important subject of concern being discussed by the HPV vaccine research community,[46,47] there was no evidence of HR HPV type replacement in any of the pre-licensure HPV vaccine clinical trials.

The U.S. CDC-sponsored National Health and Nutrition Examination Survey (NHANES) has collected and analyzed HPV DNA vaginal swab samples on over 8,000 females between 2003 and 2010. NHANES HPV DNA Vaginal Swab Survey data are ideal for capturing temporal trends in HPV genotypic prevalence. Combining the first two 2-year surveys—2003-2004 and 2005-2006—can serve as a pre-vaccine era period of unvaccinated/unexposed females, while combining the latter two 2-year surveys—2007-2008 and 2009-2010—serves as the post-vaccine era period representing the general U.S. female population during the first four years of widespread HPV vaccination. No other population-based cross-sectional survey has socio-demographic, sexual behavioral and laboratory data from the pre- and post-vaccine eras.

An analysis of the HPV DNA Vaginal Swab Surveys was conducted to assess the relationship between vaccination and the beneficial outcome of cross-protection (decreased prevalence) of non-vaccine-targeted HR HPV genotypes from the α7 and α9 species. This is the first study to examine for cross-protection of individual HR HPV genotypes from the α7 and α9 species in multiple age groups. This exploration is necessary to determine if the genotypic cross-protection documented in the initial HPV vaccine clinical trials now exists at the population level.

In addition to an examination of HR HPV genotypic cross protection, I tested for type-replacement—increased prevalence of combined non-vaccine-targeted HR HPV and those from the α-7 and α-9 species. It might be too early to see evidence of viral type-replacement while still in the first decade of HPV vaccination. Nevertheless, examining this effect at the population level is prudent as it would provide public health officials with a robust sense of urgency for continuing to recommend routine cervical cancer screening in HPV-vaccinated females.

Besides temporal trends analyses comparing non-vaccine-targeted HR HPV genotypic prevalence, I attempted to determine if there are geographical differences in genotypic prevalence in the post-vaccine era based on vaccine uptake. The results of this geographical ecological analysis augment the pre- and post-vaccine prevalence comparison, offering additional HPV vaccine post-licensure indicator information of virologic cross-protection or type-replacement. This analysis is novel in that no published data exist on the geographic correlation of U.S. HPV vaccination rates and genotypic prevalence.

Unlike previously published ecological studies examining temporal trends in vaccine-type HPV genotypic prevalence in young females aged 14 to 19 years,[48-50] my investigations

included females up to age 59 years. I also examined whether socio-demographic characteristics such as age, race/ethnicity, and education as well as known HPV risk factors, including sexual activity and marital status,[51-54] confound prevalence estimate comparisons.

4.2 Methods

Study and collection of data

NHANES is the largest of the four major CDC-sponsored National Center for Health Statistics (NCHS) data collection programs. The initial basis for NCHS surveys was the National Health Survey Act (P.L.84–652), enacted on July 3, 1956.[55]

The NHANES program commenced in the early 1960s, conducting a series of surveys focusing on different population groups and health topics. It became a continuous survey program in 1999 that focused on a variety of health and nutrition measurements of a nationally representative sample of approximately 20,000 persons each year. NHANES obtains a nationally representative sample of non-institutionalized US individuals by using a complex, stratified, multistage probability sample design with unequal probabilities.[56] Adolescents, non-Hispanic blacks, and Mexican Americans are oversampled in order to allow sufficient sizes for subgroup analysis.

HPV DNA testing for females was added to NHANES in its 2003-2004 survey.[51 51] HPV DNA testing occurred on females aged 14-59 years with the use of self-collected vaginal swabs. Consenting participants have a household interview followed by a physical examination in a mobile examination center (MEC). MECs are made up of four sideways-linked trailers (similar to a mobile home) which resemble a fully-functioning medical clinical with myriad diagnostic

equipment (e.g., CT scan), and banks of computers for data entry. They are situated in locations convenient for enrolled participants.

Sample for analysis

In each of the four cycles (2003-2004; 2005-2006; 2007-2008; 2009-2010), the CDC's NCHS enrolled approximately 2,500 females for its NHANES HPV Vaginal Swab Surveys. Final data available for HPV DNA analysis usually drops to 2,100 females for a number of reasons, including 1) refusal of examination in the MEC; 2) unwillingness to self-collect a cervicovaginal swab sample; and 3) inadequacy of samples for DNA typing. Data from 9, 850 females aged 14-59 years are available for my analysis from the four 2-year NHANES HPV Vaginal Swab Surveys. All data are publicly available for downloading on the CDC's NHANES website, except for those pertaining to female minors aged 14-17 years. Data on minors, termed "limited used data," are only available for analyses after a research proposal has been approved by the NCHS.

Specimen collection

The protocol detailing instructions and methods of specimen collection has been described elsewhere.[51] In brief, self-collection of a cervicovaginal sample took place in private in the bathroom of a MEC. Females were given a collection device that had a small foam swab on a plastic handle packaged in an individual resealable plastic sleeve (Catch-All Sample Collection Swabs Epicenter, Madison, WI). Foam swabs were to be inserted into the vagina—similar to inserting a tampon—gently turned for 10 seconds and then replaced into the plastic sleeve. NHANES personnel collected the material and mailed it to a CDC laboratory for

processing. Detailed specimen processing instructions are outlined in the NHANES Laboratory Procedure Manual.

Laboratory methods

DNA extractions were performed within one month of collecting samples employing a modified QIAmp Mini Kit.[51,57] The extract (100 µL total volume) was either tested immediately or stored at –20C. To serve as a contamination control, a water blank was processed through all steps of extraction for every 40 samples.

DNA from the vaginal swab was extracted using two assays, the Qiagen Hybrid Capture (HC2) and Roche Linear Array (LA). HC2 is a nucleic acid hybridization microplate assay with signal amplification. It uses chemiluminescence for the qualitative detection of eighteen types of HPV DNA in cervical specimens. The hc2 dichotomously differentiates between the two HPV DNA groups: low-risk (LR) HPV Types: 6, 11, 42, 43, and 44; and HR HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. It is, however, unable to determine the "specific" HPV genotype present.

The Roche LA is based on HPV L1 consensus polymerase chain reaction (PCR) with biotinylated PGMY09/11 primer sets. It also includes biotinylated β-globin primers as an internal control for sample amplification. The primer mix amplifies essentially all HPV types that are found in the genital tract along with the human β-globin.[58] All samples are hybridized to the typing strip which included probes for 37 HPV types: HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39). The Roche LA is considered "research only" because it has not been

approved by the FDA for commercial use in the U.S. It is, however, approved for commercial use in the European Union.

The 2003-2004 vaginal Swab Survey initially analyzed specimens using the Roche prototype line blot assay. The second and following NHANES cycles discontinued the Roche prototype line blot assay and employed the Roche Linear Array Assay (LA). Saved samples from the 2003-2004 Survey were re-analyzed with the Roche LA, thus allowing for analyses on four 2-year surveys using the same laboratory methods.

Demographic and behavioral data of study subjects

Before all female participants aged 14-59 years enter the MEC to self-collect vaginal swab samples, household interviews are conducted to obtain demographic information, including age, race, ethnicity, education, and marital status. Race and ethnicity were self-reported into categories, including non-Hispanic black, non-Hispanic white, and Mexican American. Using audio computer-assisted self-interview (ACASI), participants self-reported sexual history information. Sex was defined as vaginal, oral, or anal sex. For those females aged 14-17 years who reported sexual activity, additional questions about sexual behavior were asked, such as age at first sex and number of lifetime partners. For the females aged 18 years or older who reported past sexual activity, they were asked additional questions on the number and gender of sex partners in the last 12 months, lifetime sex partners, and past history of sexually transmitted infections.

Categorization of non-vaccine-targeted HR HPV

In all analyses, non-vaccine-targeted HR HPV is defined as a combination of the following 18 HPV genotypes: HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82. Of these 18 non-vaccine-targeted HR HPV genotypes, nine are further categorized according to their familial relationship with the α7 species (HPV 39, 45, 59, and 68) or the α9 species (HPV 31, 33, 35, 52, and 58).

HPV prevalence data aggregated by state

In an attempt to determine if there is HPV cross-protection or type-replacement based on geographical differences in vaccine uptake, I compared the prevalence of non-vaccine-targeted HR HPV in the top-10 states with the highest vaccination coverage with that of the bottom-10 states with the least vaccine coverage in the post-vaccine period (2007-2010). To ascertain if the change in non-vaccine-targeted HR HPV genotypic prevalence is novel in the post-vaccine era, I compared the baseline prevalence in the aggregated states from the first two 2-year pre-vaccine era surveys.

States have been categorized based on published data from the adolescent portion of the CDC-sponsored National Immunization Survey, NIS-Teen.[59] NIS-Teen is considered the most accurate of all immunization surveys because of its use of physician verification.[60] The top-10 states have a median of 44.8% HPV vaccine coverage for all 3 doses while the bottom-10 states have a median of 22.5%. The top-10 states in descending order of most HPV vaccine coverage are: Rhode Island; South Dakota; Massachusetts; Connecticut; Washington; Wisconsin; Nebraska; New Hampshire; Pennsylvania; Virginia. The bottom-10 states in ascending order

with least vaccine coverage are: Idaho; Arkansas; Mississippi; Alabama; Utah; Georgia; Indiana; Florida; Alaska; Kansas. (See **Figures 4.2a & 4.2b** for details).

Categorization of confounding variables

Potential confounders to be included in the pre-vaccination/post-vaccination analyses are: 1) age; 2) race/ethnicity; 3) education; 4) marital status; 5) ever having sex; 6) age at sexual debut; and 7) total number of lifetime male sex partners. Age is categorized as: 14-19 years; 20-24 years; 25-29 years; 30-39 years; 40-49 years; and 50-59 years. Age at sexual debut is categorized as younger than 16 years of age, older than 16 years of age, or never having had sex. (See **Table 4.2** for a detailed listing of variables and their categorizations.)

Statistical Analysis

All analyses were conducted using NHANES 2003-2010 sample weights to account for the complex survey design and to provide unbiased estimates of HPV prevalence and predictors for the total US population.[61,62] Four-year weights are provided and recommended by NHANES when combining two 2-year surveys. Data already account for weighting and a complex sample design, where WTMEC4YR = ½ x WTMEC2YR for a 4-year sample.

Since the sample weights in NHANES were employed to oversample certain groups in an attempt to match the demographics of the U.S., I adjusted for strata and cluster to create unbiased estimates of my standard errors (e.g., 95% confidence intervals and p-values). The primary analyses compared non-vaccine-targeted HR HPV genotypic prevalence in females in the pre-vaccine period (2003-2006) and post-vaccine period (2007-2010) samples across the 50 U.S. states, with the latter being further stratified into two groups based on the aggregated ten states

with the most vaccination coverage and least vaccination coverage as per CDC-sponsored NIS-Teen Survey data.

Prevalence estimates of HPV DNA are reported as percentages with 95% confidence intervals. Confidence intervals for HPV type-specific prevalence were estimated using methods adopted by Korn and Graubarde for proportions with small expected number of positive counts in complex multicenter survey data.[63]

To assess for differences in proportions of females testing HPV DNA-positive, $\chi 2$ tests and student t-tests were used. The student t-test assumes that the data has a normal distribution, and that the covariance is small. The CDC's National Center for Health Statistics (NCHS) encourages use of the student t-test for NHANES data to detect differences in health outcomes or risk factors between subpopulations, and it contends that NHANES data "do meet both assumptions" provided data are not divided into very small sub-domains. To explore for confounding, bivariate analyses with demographic and behavioral characteristics were conducted using a survey design-adjusted Wald F test.

I employed "modified Poisson" regression, as outlined by Zou,[64] to adjust comparisons of prevalence by socio-demographic characteristics that were found to vary between the groups of females and report adjusted prevalence ratios (aPR). Modified Poisson regression for binary outcomes in population surveys provides robust error variance estimation and creates 95% confidence intervals with the correct coverage.[65] Moreover, the robust error variances can be attained by using the repeated statement along with the subject identifier even when there is only one observation per subject. Statistical significance was tested at the level of $P< 0.05$. Associations were considered significant if the P value for the Satterthwaite adjusted F test[66] is

<0.05, and those variables were retained in the main effects model. The formula for Satterthwaite's approximation for the degrees of freedom for the approximate t statistic is: df = [((w1+w2)2)/(([(w12)/(n1-1)]+[(w22)/(n2-1)]))].[67] All analyses were be conducted using SAS version 9.4.[68]

4.3 Results

Comparison of non-vaccine-targeted HR HPV prevalence between 2003–2006 and 2007–2010

A total of 9,850 females between the ages of 14-59 years were included in the analyses: 4,990 from the first two 2-year surveys of 2003-2004 and 2005-2006 (henceforth referred to as the pre-vaccine period) and 4,860 from the latter two 2-year surveys of 2007-2008 and 2009-2010 (henceforth referred to as the post-vaccine period). **Table 4.3** documents the age distribution of females enrolled in the pre- and post-vaccine periods. Due to NHANES's discontinuation of over-sampling younger females after the 2005-2006 Vaginal Swab Survey, the most significant demographic difference between females in the two periods is the approximate 50% reduction in the number of females aged 14-19 years. The pre-vaccine period consisted of 1,660 females compared to 887 in the post-vaccine period. However, the weighted distribution of females in this age category is not significantly different: 13.4% compared to 13.0%.

Table 4.4 details the difference in all non-vaccine-targeted HR HPV and α7 and α9 species prevalence among the entire sample of females between the pre- and post-vaccine periods. No significant unadjusted or adjusted difference was observed in all non-vaccine-targeted HR HPV and α7 species. There was a decrease observed in α9 species prevalence in the post-vaccine period from 7.7% to 6.4% (PR = 0.83; 95% CI: 0.70-0.99). However, after

adjusting for socio-demographic variables and sexual behavior, this decreased prevalence no longer remained statistically significant (aPR = 0.84; 95% CI: 0.69-1.03).

When stratified by age, as documented in **Table 4.5**, the only statistically significant prevalence decrease in the post-vaccine period was found for combined non-vaccine-targeted HR HPV in the youngest age group. In adolescents aged 14-19 years, combined non-vaccine-targeted HR HPV decreased from 19.8% (95% CI: 17.3-22.3) to 15.6% (12.6-18.5) with a PR of 0.79; (95% CI: 0.63-0.99). The only significant prevalence increase documented in all age groups was for combined non-vaccine-targeted HR HPV in females aged 25-29 years, from 23.1% (95% CI: 18.5-27.7) to 32.3% (27.3-37.4) with a PR of 1.40 (95% CI: 1.09-1.80).

Comparison of individual HPV genotypic weighted prevalence between vaccine periods by age

As documented in **Table 4.6a-4.6f,** there was no statistically significant change observed in grouped or individual HPV genotypic prevalence of α7 or α9 species between the two vaccine periods in any of the age groups.

Weighted HPV prevalence among females ages 14-29 years according to vaccination status

Self-reported vaccine history data exists on 1,835 of females aged 14-29 years enrolled in the post-vaccine period NHANES Vaginal Swab Surveys. NHANES defines "vaccinated" as receiving ≥1 dose of either the bivalent or quadrivalent HPV vaccine. As documented in **Table 4.7**, there was a statistically significant higher prevalence of combined non-vaccine-targeted HPV in vaccinated females compared to those not vaccinated. Non-vaccine-targeted HPV genotypic prevalence was 30.3% in those who were vaccinated and 23.9% in non-vaccinated

females with a PR of 1.27 (95% CI: 1.02-1.52). Alpha-7 species prevalence was 13.4% in vaccinated females compared to 8.5% in those unvaccinated (PR = 1.58; 95% CI: 1.15-2.01). There was a trend towards greater α9 species prevalence in vaccinated females: 10.8% versus 7.6% (PR = 1.43; 95% CI: 0.97-1.90). It is important to note that for these observations, the prevalence estimates had a relative standard error ≥30%, and thus, results are considered unstable and should be interpreted with caution.

Geographical analysis of HR HPV prevalence in states with high-and low-vaccine coverage

Figures 4.1a and 4.1b detail 2010 NIS-Teen Survey data on the top-10 states with the highest reported HPV vaccine uptake—defined as receiving all 3 recommended doses—and the bottom-10 states with the lowest reported vaccine coverage. The top-10 states had a median 44.8% coverage (range: 41.5%-55.1%), while the bottom-10 states had a median 22.5% coverage (range: 17.6%-25.1%).

The aggregated states were compared in the 4-year pre-vaccine period in order to determine baseline non-vaccine-targeted HR HPV prevalence before the advent of HPV vaccination. As detailed in **Table 4.8a,** there was a small and insignificant non-vaccine-targeted HR HPV prevalence decrease during the pre-vaccine period in the top-10 states with the highest reported vaccine coverage: 20.1% versus 19.1% (p=0.73). **Table 4.8b** documents non-vaccine-targeted HR HPV prevalence between both sets of aggregated states in the post-vaccine period. During this period, no appreciable difference in non-vaccine-targeted HR HPV prevalence was observed. Non-vaccine-targeted HR HPV prevalence in females residing in the top-10 states was 22.1% compared to 21.9% for those residing in the bottom-10 states (p=0.96). This observation of no difference in non-vaccine-targeted HR HPV prevalence in females living in geographical

areas where HPV vaccination is more common suggests no evidence of genotypic cross-protection or type-replacement.

Risk factors associated with non-vaccine-targeted HR HPV prevalence in females from both periods

Table 4.9a documents the socio-demographic and sexual behavior variables which were associated with combined non-vaccine-targeted HR HPV infection in all females from both the pre- and post-vaccine periods. In the multi-level GEE model, increased risk of infection with non-vaccine-targeted HR HPV was associated with younger age—females aged 20-29 years; race/ethnicity (non-Hispanic black and Mexican America); lower education status (e.g., less than high school graduate or only a high school graduate); not being married, be it single, partnered, or widowed/divorced/separated; ever having sex (regardless of age at sexual debut); and having two or more lifetime sexual partners. Interestingly, the only variable found to have a protective effect was having one lifetime sexual partner compared to the referent group of no lifetime sexual partners (risk ratio = 0.40; p-value <0.01).

Only socio-demographic variables were associated with increased risk of infection with HPV genotypes from the α7 and α9 species. As detailed in **Tables 4.9b and 4.9c**, none of the sexual behavior variables conferred an increased risk or a protective effect. And in contrast to the GEE model for non-vaccine-targeted HR HPV, non-Hispanic black race did not reach statistical significance as a risk factor for infection with α7 or α9 species genotypes.

4.4 Discussion

We found a marked decrease in combined non-vaccine-targeted HR HPV prevalence in females under the age of 20 years between the pre- and post-vaccine periods from the NHANES Vaginal Swab Surveys. The 21% decrease observed in non-vaccine-targeted HR HPV prevalence—irrespective of vaccination status—in 887 adolescent females from the post-vaccine period is highly suggestive of genotypic cross-protection.

This statistically significant decrease is novel and differs from three published cross-sectional ecological studies with similar methods to ours.[48-50] Two ecological studies, from the U.S.[48] and Australia,[49] documented a sizable prevalence decrease of non-vaccine-targeted HR HPV in adolescent females; however, neither reached statistical significance. In contrast, a 2012 U.S. study found statistically significant increased prevalence of non-vaccine-targeted HR HPV in their entire sample of post-vaccine era females aged 13-26 years.[50]

It is important to note that when we narrowed our unit of analysis from the 18 combined non-vaccine-targeted HR HPV genotypes to their respective alpha species (α7 and α9) and further by individual genotypes, only modest, non-statistically significant decreases in prevalence were observed. With such limited observations and small cells, this was understandably a power issue.

Of all individual non-vaccine-targeted HR HPV genotypes, we were expecting to observe a significant post-vaccine period decrease in HPV 33, a genotype phylogenetically related to HPV 16. Marked decreased prevalence in HPV 33—suggesting cross-protection—was observed in the original Gardasil[37] and Cervarix[39] phase 3 pivotal trials and more recently in an ecological study from Australia.[69] It is possible that there was a significant decrease of HPV 33 prevalence

in our sample of post-vaccine period females; however, the CDC's NCHS suppressed results in the dataset because of <10 observations and a relative standard ≥30%. Results in the dataset were also suppressed for HPV 45 prevalence in post-vaccine period females.

Limited self-reported data are available on the particulars of HPV vaccination status of females in the 2007-2008 and 2009-2010 NHANES HPV DNA Vaginal Swab Surveys. Vaccine information, in the form of two questions—received the vaccine (yes or no) and number of doses—is the extent of the information from females in the Vaginal Swab Surveys who answered the NHANES Immunization (IMQ_E and IMQ_F) questionnaire.

As expected, history of vaccination was inversely correlated with age: 34.1%, 15.8%, and 7.4% of females aged 14-19, 20-24, and 25-29 years, respectively. Of those reporting receipt of ≥1 dose, 62.5%, 43.2%, and 45.7% said they received all 3 recommended doses. The 34.1% rate of receiving ≥1 dose documented in the NHANES adolescents is considerably lower than the national average. The physician-verified CDC-sponsored NIS-Teen Survey reported that 48.7% adolescent females revived ≥1 HPV vaccine dose in 2010.[59]

The significant increased prevalence of combined non-vaccine-targeted HR HPV as well as α7-species in females who received ≥1 vaccine dose compared to those unvaccinated is suggestive of type-replacement. These results confirm a smaller U.S. ecological study which found a 13.6% increase of combined non-vaccine-targeted HPV in vaccinated females who exclusively received Gardasil.[50] Conversely, two recently published ecological studies—from Scotland[70] and Australia[69]—observed decreased prevalence in a combination of HPV 31, 33, and 35 in vaccinated females compared to those unvaccinated from the post-vaccine period. Of note, the Scottish study only included women who had received the bivalent vaccine, Cervarix.

No significant difference in weighted socio-demographic variables or reported sexual behavior was observed at baseline between the pre- and post-vaccine period females. In the GEE multivariate analyses of risk for combined non-vaccine-targeted HR HPV prevalence, all variables—younger age, lower education level, non-white race/ethnicity single marital status, and sexual activity—were significantly associated with increased risk. It is highly likely that both sexual behavior variables failed to reach statistical significance in the α7 and α9 species GEE models because of insufficient power to detect a difference.

In numerous longitudinal studies, younger age has been associated with increased risk of HPV.[51,71,72] Our finding that single marital status is associated with increased risk of HPV confirms a number of previously published studies.[73-75]

There are conflicting data that suggest non-white race is independently associated with LR and/or HR HPV DNA. Our results confirm results from two large studies documenting increased risk of HPV infection in non-Hispanic black females.[76,77]

We are uncertain as to why there was no appreciable difference in non-vaccine-targeted HR HPV prevalence between the aggregated top-10 and bottom-10 states in HPV vaccine coverage. While data are available from the 2010 NIS-Teen Survey on vaccine coverage of the top-10 states and the bottom-10 states, the CDC's NCHS does not allow knowledge of exact geographic location (e.g., state) of study participants due to patient confidentiality concerns. The smallest unit of analysis the NCHS allows is the 10-state aggregation and a proviso that participants simply "resided in one of the ten states." Thus, it remains unclear how well distributed the post-vaccine era females were geographically.

Strengths & Limitations

A significant strength of these analyses comes from access to publicly available NHANES data with its many years of well-characterized sampling method protocols and quality-control laboratory manuals. NHANES use of weighting and over-sampling of African Americans, Asians, and Hispanics speaks to its rigorous inclusion of myriad U.S. and foreign-born populations in order to achieve a nationally representative sample.

The HPV DNA Vaginal Swab Survey, like many others in NHANES, is unique in that it combines interviews, physical examinations, and specimen collection. No other population-based survey in the U.S. or internationally has continuously collected HPV DNA genotypic data along with risk factor information including socio-demographic information, lifestyle, and sexual behavior history.

An additional strength of the analyses is that it is the first reported comparison of individual and grouped vaccine-type HPV DNA genotypic prevalence in a wide age range of females from 14 to 59 years before and after the advent of HPV vaccination in 2006. Likewise, this is the first reported geographic analysis of HPV prevalence to be conducted in the history of the NHANES Vaginal Swab Surveys.

There are also a number of limitations to these analyses. By their nature, the use of population-level data rather than individual-level data in these ecological analyses hampered our ability to assess causation between exposure and outcome. While there are specific data on HPV genotype prevalence from the pre- and post-vaccination periods, no concrete, physician-verified data exists to determine if these females in the latter period were actually vaccinated. It is only self-report data.

My geographical comparator groups—the top-10 and bottom-10 states in vaccine coverage—can be considered crude and somewhat arbitrary. As noted, this was the lowest geographic unit of analysis the CDC's NCHS would make available. Nonetheless, the use of the CDC-sponsored NIS-Teen Survey for quantifying vaccine coverage by state is considered the most accurate of all immunization surveys because of its use of physician verification.[60]

While NHANES strives to conduct its samples with rigor and has excellent laboratory quality control, there are no other evaluations in the general U.S. population using self-collected vaginal swabs that would have allowed for a direct comparison of past or present HPV DNA prevalence. Also, NHANES data only offer HPV DNA point prevalence. Because HPV DNA genotypes often clear in females in 6-12 months,[78] this point prevalence is certain to underestimate cumulative incidence. Moreover, the HPV DNA vaginal swab sampling only measures current infection and does not indicate past exposure (and thus clearance) to HPV. This, however, is neither the fault of NHANES sampling nor its laboratory assay. No assay currently exists to identify past/cleared HPV infection, the duration of given infection, or if a particular HPV genotype is either new or a reinfection.

Another limitation is the inability to control for confounding of vaccine uptake in males in the two 10-state comparison groups. While Merck did received an additional indication from the FDA for Gardasil to be administered to males aged 9-26 years in 2009, data from 2011 NIS-Teen survey on HPV vaccination coverage in males documents a national average of only 8.3% in boys aged 13-17 years.[79] Of note, only 19 states reported HPV vaccination coverage in males to CDC's 2011 NIS-Teen survey. With male HPV vaccine coverage extremely low in states and/or not reported, it was not feasible to control for male HPV vaccination.

Public Health Significance

Our analyses of temporal trends in non-vaccine-targeted HR HPV prevalence between the pre- and post-vaccine periods were in response to the CDC's statement that observing early virological outcomes of HPV vaccination is a "critical aspect of monitoring its population impact."[80]

It is, of course, too early to conclude if HPV vaccination in females has had an impact in preventing ICC at the population levels. The marked decrease of HPV 16 and 18 observed in an assortment of international ecological studies comparing females from the pre- and post-vaccine period is reassuring.[48-50,69,70] However, the suggestion of genotypic type-replacement with increased prevalence of non-vaccine-targeted HR HPV in our sample of vaccinated females—which was found in previously published smaller ecological study—is concerning.[50]

In both studies, the genotypic type-replacement appears to be the result of receiving the first generation Gardasil. The smaller 2012 ecological study enrolled women from clinics which exclusively administered Gardasil. In our 2007-2010 post-vaccine period sample, we are certain that a vast majority of the females with a history of vaccination received Gardasil, essentially because Cervarix was not FDA-approved until October 2009.

The clinical significance of this possible genotypic type-replacement is unknown, especially with the extremely long latency period between persistent HR HPV infection, high-grade CIN, and then ICC. Likewise, genotypic type-replacement with non-vaccine-targeted HR HPV may be somewhat of a moot point with the advent of Gardasil-9. For females—and possibly males—who are beginning to receive Gardasil-9 with its nonavalent structure, a discussion of HR HPV cross-protection or type-replacement is redundant. It is, however, not

redundant for individuals who have received over 200 million doses of the original Gardasil since 2006.[81] Moreover, of the five additional genotypes, only one, HPV 45, is from the α7 species in which we found the strongest evidence of grouped type-replacement. Other HR HPV genotypes from the α7 species—HPV 39, 59, and 68—should now be a cause of concern.

Conclusion

The present research adds to the accumulating body of evidence that HPV vaccination in adolescent females not only protects against HPV 16 and 18, but confers additional virologic activity that is both advantageous with cross-protection and deleterious with type-replacement. We specifically documented decreased prevalence of combined non-vaccine-targeted HR HPV in adolescent females irrespective of vaccination status, but found also a marked increase in the same HR HPV types in vaccinated females up to age 29 years.

Further study is warranted to confirm our observation of increased prevalence of non-vaccine-targeted HR HPV in vaccinated females who received the original Gardasil. HPV DNA cross-sectional studies could be undertaken in Australia and European countries with vaccine registries and cervical cancer screening programs. If pre- and post-vaccine era cohorts do not exist, it is possible that pre-vaccine era HPV DNA prevalence data (or saved vaginal swab samples) could serve as historical controls. It would also be prudent to ascertain if there is increase prevalence of non-vaccine-targeted HR HPV in colposcopy tissue samples in women with high-grade CIN.

Finally, this possibility of type-replacement with carcinogenetic HR HPV genotypes adds emphasis to the American College of Obstetricians and Gynecologists (ACOG) cervical cancer

screening guidelines which state: “Women who have received the HPV vaccine should be screened according to the same guidelines as women who have not been vaccinated.”[82]

4.5 Table & Figures

Table 4.1. Studies classifying HPV genotypes as "high risk" between 1995 and 2009

HPV Genotype / Study	16[a,b]	18[a,c]	26	31[b]	33[b]	35[b]	39[c]	45[c]	51	52[b]	53	56	58[b]	59[c]	66	67	68[c]	70	73	82
Jacob, 1995[83]																				
Gravitt, 1998[84]																				
Davies, 2001[85]																				
van den Brule, 2002[86]																				
Munoz, 2003[13]																				
Cogliano, 2005[14]																				
Smith, 2007[87]																				
Bosch, 1995[88]																				
Bouvard, 2009[15]																				

[a] HR vaccine type: HPV 16 and/or 18
[b] Alpha-9 species: HPV 16, 31, 33, 35, 52, and 58
[c] Alpha-7 species: HPV 18, 39, 45, 59, and 68

Sufficient evidence for cervical cancer

Probably carcinogenic

Possibly carcinogenic

Table 4.2. Demographics: weighted distribution of potential confounders in the pre- and post-vaccination periods

Variables	Categorization	2003-2006 (N=4990) %	2007-2010 (N=4860) %
Age	14-19 years	13.4	13.0
	20-24 years	11.1	10.5
	25-29 years	9.8	10.9
	30-39 years	21.6	20.8
	40-49 years	24.5	23.5
	50-59 years	19.7	21.3
Race/ethnicity	Non-Hispanic white	67.7	64.6
	Non-Hispanic black	13.4	13.2
	Mexican American	8.7	9.2
	Other	10.1	13.0
Education[a]	Less than high school	14.3	17.7
	High school graduate	23.3	21.5
	More than high school	62.4	60.7
Marital status	Married	49.6	54.7
	Widowed/divorced/separated	14.3	15.9
	Never married	28.7	20.8
	Living with partner	7.4	8.6
Age at sexual debut	<16 Years	39.4	40.6
	>16 Years	56.5	55.5
	Never had sex	4.2	3.9
Total lifetime sex partners	0	0.4	2.3
	1	18.0	16.3
	2	10.1	10.2
	3-5	28.7	29.4
	≥6	42.8	41.7

Table 4.3. Age distribution of females between the pre- and post-vaccine periods

Age Group	2003-2006 (N=4,990)	2007-2010 (N=4,860)
14-19 years	N=1,660	N=887
20–24 years	N=551	N=513
25–29 years	N=523	N= 472
30–39 years	N=850	N= 1,034
40–49 years	N=795	N= 1,072
50–59 years	N=611	N= 882

Table 4.4. Non-vaccine-targeted HR HPV type prevalence among the entire sample of females

HPV Type	Prevalence % (95% CI)		Unadjusted Prevalence Ratio[a] (95% CI)	Adjusted[b] Prevalence Ratio[a] (95% CI)
	2003-2006 (N=4990)	2007-2010 (N=4860)		
HR non-vaccine type[c]	23.0 (21.5-24.5)	22.1 (20.7-23.5)	0.96 (0.88-1.1)	0.98 (0.88-1.08)
Alpha-9 species[d]	7.7 (6.8-8.7)	6.4 (5.6-7.2)	0.83 (0.70-0.99)*	0.84 (0.69-1.03)
Alpha-7 species[e]	7.4 (6.41-8.37)	7.5 (6.56-8.46)	1.01 (0.85-1.22)	1.02 (0.83-1.24)

[a] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance[64]

[b] Adjusted for age, race/ethnicity, education, marital status, age at sexual debut, and number of total lifetime sexual partners

[c] HR non-vaccine HPV types combined (n=18): HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)

[d] Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)

[e] Alpha-7 species combined: HPV 39, 45, 59, and 68 (excluding HPV 18)

* P<0.05

Table 4.5. Non-vaccine-targeted HR HPV prevalence in females stratified by age group

Age	HPV Type	2003-2006 (% & 95% CI)	2007-2010 (% & 95% CI)	Prevalence Ratio[f] (95% CI)
14-19 y/o		*n= 1660*	*n= 887*	
	HR non-vaccine type[b]*	19.8 (17.3-22.3)	15.6 (12.6-18.5)	0.79 (0.63-0.99)
	Alpha-9 species[c]	6.9 (5.4-8.4)	5.2 (3.4-7.0)	0.76 (0.50-1.13)
	Alpha-7 species[d]	11.8 (8.4-15.2)	9.9 (5.6-14.2)	0.84 (0.50-1.41)
20-24 y/o		*n=551*	*n=558*	
	HR non-vaccine type	32.8 (27.9-37.7)	37.8 (32.7-42.8)	1.15 (0.94- 1.40)
	Alpha-9 species	13.6 (10.1-17.1)	12.3 (9.2-15.5)	0.91 (0.63-1.30)
	Alpha-7 species	12.6 (9.1-16.1)	15.22 (11.5-18.9)	1.21 (0.83-1.75)
25-29 y/o		*n=523*	*n=540*	
	HR non-vaccine type*	23.1 (18.5-27.7)	32.3 (27.3-37.4)	1.40 (1.09-1.80)
	Alpha-9 species	8.4 (5.3-11.5)	10.2 (7.1-13.2)	1.21 (0.75-1.94)
	Alpha-7 species	9.8 (6.6-13.0)	9.1 (5.9-12.3)	0.93 (0.58-1.50)

Age	HPV Type	2003-2006 (% & 95% CI)	2007-2010 (% & 95% CI)	Prevalence Ratio[a] (95% CI)
30-39 y/o		*n=850*	*n=1,062*	
	HR non-vaccine type	23.5 (20.0-26.9)	21.3 (18.5-24.0)	0.91 (0.75-1.10)
	Alpha-9 species	7.3 (5.2-9.3)	5.1 (3.7-6.5)	0.70 (0.47-1.04)
	Alpha-7 species	7.7 (5.5-9.8)	6.9 (5.2-8.6)	0.90 (0.62-1.30)
40-49 y/o		*n=795*	*n=1,164*	
	HR non-vaccine type	22.4 (19.1-25.8)	20.5 (17.5-23.5)	0.91 (0.74-1.26)
	Alpha-9 species	7.0 (5.0-9.0)	4.9 (3.3-6.4)	0.70 (0.46-1.06)
	Alpha-7 species	5.6 (3.8-7.3)	6.6 (4.7-8.4)	1.18 (0.78-1.19)
50-59 y/o		*n=611*	*n=888*	
	HR non-vaccine type	19.7 (16.2-23.3)	15.8 (12.7-18.8)	0.80 (0.61-1.04)
	Alpha-9 species	6.0 (3.9-8.0)	5.3 (3.4-7.1)	0.88 (0.54-1.44)
	Alpha-7 species	4.3 (2.5-6.0)	4.1 (2.4-5.7)	0.96 (0.54-1.70)

[a] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance[64]

[b] HR non-vaccine HPV types combined ([n=18] HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)

[c] Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)

[d] Alpha-7 species combined: HPV 39, 45, 59, and 68 (excluding HPV 18)

* P<0.05

Table 4.6a. Weighted prevalence of HPV alpha 9 & 7 species among all females

	2003-2006 (n=4,990)			2007-2010 (n=4,860)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	N=438	7.7	(6.8-8.7)	N=357	6.4	(5.6-7.2)
Individually:						
31	N=133	2.2	(1.7-2.7)	N=76	1.6	(1.1-2.0)
33	N=56	1.5	(1.0-1.9)	Suppressed: RSE of ≥30% or <10 observations		
35	N=64	1.3	(0.8-1.7)	N=81	1.7	(1.2-2.1)
52	N=177	3.5	(2.8-4.2)	N=143	3.1	(2.5-3.7)
58	N= 83	1.8	(0.9-1.7)	N=84	1.4	(1.0-1.8)
Alpha-7 species						
Combined:	N=412	7.1	(6.2-8.0)	N=368	7.1	(6.2-8.0)
Individually:						
39	N=122	2.2	(1.7-2.7)	N=113	2.7	(2.1-3.3)
45	N=105	2.0	(1.5-2.5)	Suppressed: RSE of ≥30% or <10 observations		
59	N=151	2.9	(2.3-3.6)	N=123	2.3	(1.8-2.8)
68	N=81	1.6	(1.1-2.1)	N=94	2.0	(1.5-2.5)

RSE: Relative Standard Error

Table 4.6b. Weighted prevalence of HPV alpha 9 & 7 species among females aged 14-19 years

	2003-2006 (n=1,660)			2007-2010 (n=887)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	153	6.9	(5.4-8.4)	49	5.2	(3.4-7.0)
Individually:						
31	56	2.7	(1.7-3.7)	9	1.2	(0.3-2.1)
33	10	0.5	(0.1-0.8)	Suppressed: RSE of ≥30% or <10 observations		
35	20	1.1	(0.5-1.7)	8	0.7	(1.2-1.1)
52	65	3.6	(2.4-4.8)	26	3.8	(2.0-5.6)
58	37	1.8	(1.0-2.7)	9	0.8	(0.2-1.4)
Alpha-7 species						
Combined:	146	6.5	(5.1-8.0)	56	5.2	(3.5-7.0)
Individually:						
39	54	3.3	(2.1-4.5)	22	2.8	(1.2-4.3)
45	29	1.4	(0.7-2.1)	Suppressed: RSE of ≥30% or <10 observations		
59	62	3.1	(2.0-4.2)	28	3.0	(1.6-4.3)
68	27	1.1	(0.5-1.7)	15	1.6	(0.6-2.5)

RSE: Relative Standard Error

Table 4.6c. Weighted prevalence of HPV alpha 9 & 7 species among females aged 20-29 years

	2003-2006 (n=1,074)			2007-2010 (n=985)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	115	11.2	(8.8-13.6)	138	11.3	(9.3-13.4)
Individually:						
31	26	2.4	(1.2-3.5)	44	3.5	(2.3-4.6)
33	17	2.5	(1.2-3.8)	14	1.7	(0.7-2.7)
35	20	2.2	(1.1-3.3)	38	3.8	(2.5-5.1)
52	56	6.8	(4.7-8.9)	54	5.3	(3.7-6.9)
58	18	1.9	(0.8-3.0)	20	1.42	(0.8-2.1)
Alpha-7 species						
Combined:	114	11.3	(8.9-13.7)	132	12.3	(10.0-14.6)
Individually:						
39	39	4.5	(2.8-6.2)	60	6.8	(4.8-8.7)
45	23	2.6	(1.3-3.9)	20	2.0	(1.1-3.0)
59	41	5.3	(3.4-7.2)	36	3.4	(2.1-4.6)
68	18	1.8	(0.8-2.8)	26	2.8	(1.6-4.1)

Table 4.6d. Weighted prevalence of HPV alpha 9 & 7 species among females aged 30-39 years

	2003-2006 (N=850)			2007-2010 (N=1,034)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	60	7.3	(5.2-9.3)	70	5.2	(3.8-6.5)
Individually:						
31	20	2.2	(1.0-3.3)	16	1.5	(0.7-2.3)
33	8	1.0	(0.3-1.8)	8	0.5	(0.2-0.9)
35	7	1.0	(0.2-1.8)	16	1.4	(0.7-2.1)
52	17	2.9	(1.3-4.4)	28	2.4	(1.4-3.5)
58	13	1.7	(0.6-2.8)	12	0.8	(0.4-1.3)
Alpha-7 species						
Combined:	61	7.7	(5.5-9.8)	92	8.8	(6.7-10.5)
Individually:						
39	11	1.4	(0.5-2.4)	20	2.7	(1.4-4.0)
45	19	2.4	(1.1-3.6)	26	2.5	(1.4-3.5)
59	20	3.2	(1.6-4.8)	26	2.6	(1.4-3.7)
68	15	2.0	(0.9-3.2)	20	2.2	(1.2-3.2)

Table 4.6e. Weighted prevalence of HPV alpha 9 & 7 species among females aged 40-49 years

	2003-2006 (n=795)			2007-2010 (n=1,072)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	68	7.00	(5.0-9.0)	60	3.9	(2.7-5.0)
Individually:						
31	18	2.1	(0.9-3.3)	14	0.8	(0.4-1.2)
33	11	1.4	(0.4-2.4)	4	0.2	(0.0-0.5)
35	10	1.0	(0.2-1.7)	10	0.5	(0.2-0.8)
52	22	2.5	(1.3-3.8)	16	1.8	(0.7-2.8)
58	10	0.9	(0.2-1.6)	22	1.4	(0.7-2.1)
Alpha-7 species						
Combined:	58	5.6	(3.8-7.2)	66	5.6	((3.8-7.0)
Individually:						
39	11	1.3	(0.3-2.3)	12	1.2	(0.4-2.0)
45	21	1.7	(0.9-2.5)	18	1.6	(0.7-2.6)
59	18	2.0	(0.9-3.2)	22	1.9	(0.0-2.9)
68	15	1.8	(0.7-2.9)	14	1.3	(0.5-2.2)

Table 4.6f. Weighted prevalence of HPV alpha 9 & 7 species among females aged 50-59 years

	2003-2006 (n=611)			2007-2010 (n=882)		
HPV Type	**Number positive**	**Weighted %**	**(95% CI)**	**Number positive**	**Weighted %**	**(95% CI)**
Alpha-9 species						
Combined:	42	6.0	(3.9-8.0)	54	5.0	(3.4-6.8)
Individually:						
31	13	1.7	(0.6-2.8)	8	0.5	(0.1-0.9)
33	10	1.7	(0.5-2.9)	12	1.2	(0.4-2.1)
35	7	1.2	(0.2-2.3)	12	1.7	(0.5-2.9)
52	17	2.1	(0.9-3.3)	22	2.7	(1.3-4.2)
58	5	0.6	(0.0-1.1)	12	0.9	(0.4-1.4)
Alpha-7 species						
Combined:	33	4.3	(2.5-6.0)	52	4.4	(2.7-6.1)
Individually:						
39	7	1.2	(0.1-2.2)	10	0.5	(0.2-0.9)
45	13	1.7	(0.6-2.9)	6	0.9	(0.0-1.8)
59	10	1.3	(0.4-2.3)	14	1.1	(0.3-2.0)
68	6	1.0	(0.1-1.9)	24	2.5	(1.1-3.9)

Table 4.7. Weighted HPV prevalence according to vaccination status among females aged 14-29 years from the post-vaccine period (2007-2010)

HPV Type	Prevalence (%)		
	Unvaccinated N=1,423	Vaccinated[a] N=412	PR (95% CI)[b]
HR non-vaccine types[c]	23.9	30.3	1.27 (1.02-1.52)*
Alpha-9 species[d]	7.6	10.8	1.43 (0.97-1.90)
Alpha-7 species[e]	8.5	13.4	1.58 (1.15-2.01)*

Abbreviations: CI, confidence interval; PR, prevalence ratio; HR, high risk;

[a] Vaccination defined as a self-reported history of receiving ≥1 vaccine dose

[b] Exponentiated prevalence ratios calculated using Zou's "modified Poisson" regression model with a robust error variance[64]

[c] Non-vaccine-targeted HR HPV (18 genotype combined: HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, & 82)

[d] Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16).

[e] Alpha-7 species combined: HPV 39, 45, 59, and 68 (excluding HPV 18)

*p<0.05

Table 4.8a. Weighted non-vaccine-targeted HR HPV prevalence in 2003-2006 among females who resided in one of the top-10 states with the highest HPV vaccine coverage compared to those who resided in one of the bottom-10 states with lowest HPV vaccine coverage

HPV Type	Top-10 states in vaccine coverage[b] (combined) 2003-2006	Bottom-10 states in vaccine coverage[c] (combined) 2003-2006	P-value
HR non-vaccine type	19.1%	20.1%	.7304

[a] According to 2010 NIS-Teen published data

[b] Rhode Island; South Dakota; Massachusetts; Connecticut; Washington; Wisconsin; Nebraska; New Hampshire; Pennsylvania; Virginia

[c] Idaho; Arkansas; Mississippi; Alabama; Utah; Georgia; Indiana; Florida; Alaska; Kansas

Table 4.8b. Weighted non-vaccine-targeted HR HPV prevalence in 2007-2010 among females who resided in one of the top-10 states with the highest HPV vaccine coverage compared to those who resided in one of the bottom-10 states with lowest HPV vaccine coverage

HPV Type	Top-10 states in vaccine coverage (combined) 2007-2010	Bottom-10 states in vaccine coverage (combined) 2007-2010	P-value
HR non-vaccine type	22.1%	21.9%	.9615

Table 4.9a. Multivariate GEE analysis of factors associated with non-vaccine-targeted HR HPV infection[a] among females aged 20-59 from the pre- and post-vaccine periods

Variables	Categorization	Risk Ratio & (95% CI)	P-value
Age	20-24 years	1.64 (1.36-1.97)	<.0001
	25-29 years	1.35 (1.11-1.63)	<0.01
	30-39 years	1.18 (0.99-1.41)	0.06
	40-49 years	1.08 (0.91-1.29)	0.37
	50-59 years	Referent	
Race/ethnicity	Non-Hispanic white	Referent	
	Non-Hispanic black	1.15 (1.03-1.28)	0.01
	Mexican American	1.24 (1.09-1.42)	0.001
	Other	1.10 (0.94-1.30)	0.22
Education[a]	Less than high school	1.20 (1.05-1.36)	<0.01
	High school graduate	1.32 (1.17-1.49)	<.0001
	More than high school	Referent	
Marital status	Married	Referent	
	Widowed/divorced/separated	1.70 (1.47-1.97)	<.0001
	Never married	1.65 (1.43-1.90)	<.0001
	Living with partner	1.58 (1.33-1.88)	<.0001
Age at sexual debut	Never had sex	Referent	
	<16 Years	4.09 (1.49-11.26)	<0.01
	>16 Years	4.03 (1.47-11.06)	<0.01
Total lifetime sex partners	0	Referent	
	1	0.40 (0.21-0.74)	<0.01
	2	0.77 (0.42-1.43)	0.41
	3-5	1.22 (0.68-2.17)	0.51
	≥6	1.52 (0.85-2.70)	0.16

[a] Non-vaccine-targeted HR HPV (18 genotypes combined: HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)

Table 4.9b. Multivariate GEE analysis of factors associated with non-vaccine-targeted HR HPV genotypes from the alpha-9 species[a] among females aged 20-59 from the pre- and post-vaccine periods

Variables	Categorization	Risk Ratio & (95% CI)	P-value
Age	20-24 years	2.04 (1.39-2.99)	<.001
	25-29 years	1.51 (1.02-2.23)	0.04
	30-39 years	1.12 (0.79-1.59)	0.53
	40-49 years	1.01 (0.71-1.42)	0.96
	50-59 years	Referent	
Race/ethnicity	Non-Hispanic white	Referent	
	Non-Hispanic black	1.74 (1.40-2.15)	<.0001
	Mexican American	1.45 (1.11-1.87)	<.01
	Other	1.18 (0.84-1.67)	0.34
Education	Less than high school	0.96 (0.74-1.24)	0.77
	High school graduate	1.34 (1.06-1.71)	0.02
	More than high school	Referent	
Marital status	Married	Referent	
	Widowed/divorced/separated	2.18 (1.64-2.90)	<.0001
	Never married	1.80 (1.33-2.43)	<.001
	Living with partner	1.59 (1.06-2.30)	0.03
Age at sexual debut	Never had sex	Referent	
	<16 Years	2.07 (0.35-12.23)	0.42
	>16 Years	2.39 (0.40-14.06)	0.33
Total lifetime sex partners	0	Referent	
	1	0.41 (0.12-1.43)	0.16
	2	0.78 (0.23-2.64)	0.69
	3-5	1.61 (0.62-5.09)	0.42
	≥6	1.96 (0.62-6.20)	0.25

[a] Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)

Table 4.9c. Multivariate GEE analysis of factors associated with non-vaccine-targeted HR HPV genotypes from the alpha-7 species[a] among females aged 20-59 from the pre- and post-vaccine periods

Variables	Categorization	Risk Ratio & (95% CI)	P-value
Age	20-24 years	3.13 (2.10-6.66)	<.0001
	25-29 years	2.05 (1.36-3.10)	<.001
	30-39 years	1.71 (1.18-2.49)	<.01
	40-49 years	1.26 (0.86-1.85)	0.24
	50-59 years	Referent	
Race/ethnicity	Non-Hispanic white	Referent	
	Non-Hispanic black	1.21 (0.96-1.52)	0.10
	Mexican American	1.37 (1.06-1.78)	0.02
	Other	1.14 (0.83-1.56)	0.42
Education	Less than high school	0.99 (0.76-1.29)	0.94
	High school graduate	1.30 (1.03-1.65)	0.03
	More than high school	Referent	
Marital status	Married	Referent	
	Widowed/divorced/separated	2.09 (1.55-2.83)	<.0001
	Never married	1.61 (1.22-2.13)	<.001
	Living with partner	1.40 (0.99-1.96)	0.05
Age at sexual debut	Never had sex	Referent	
	<16 Years	7.24 (1.28-41.01)	0.03
	>16 Years	6.97 (1.23-39.47)	0.03
Total lifetime sex partners	0	Referent	
	1	0.39 (0.13-1.16)	0.09
	2	0.84 (0.29-2.43)	0.74
	3-5	1.15 (0.42-3.16)	0.78
	≥6	1.50 (0.55-4.06)	0.43

[a] Alpha-7 species combined: HPV 39, 45, 59, and 68 (excluding HPV 18)

Figure 4.1a. Top-10 states with the highest estimated HPV vaccination coverage (all 3 doses) - NIS-Teen Survey, 2010

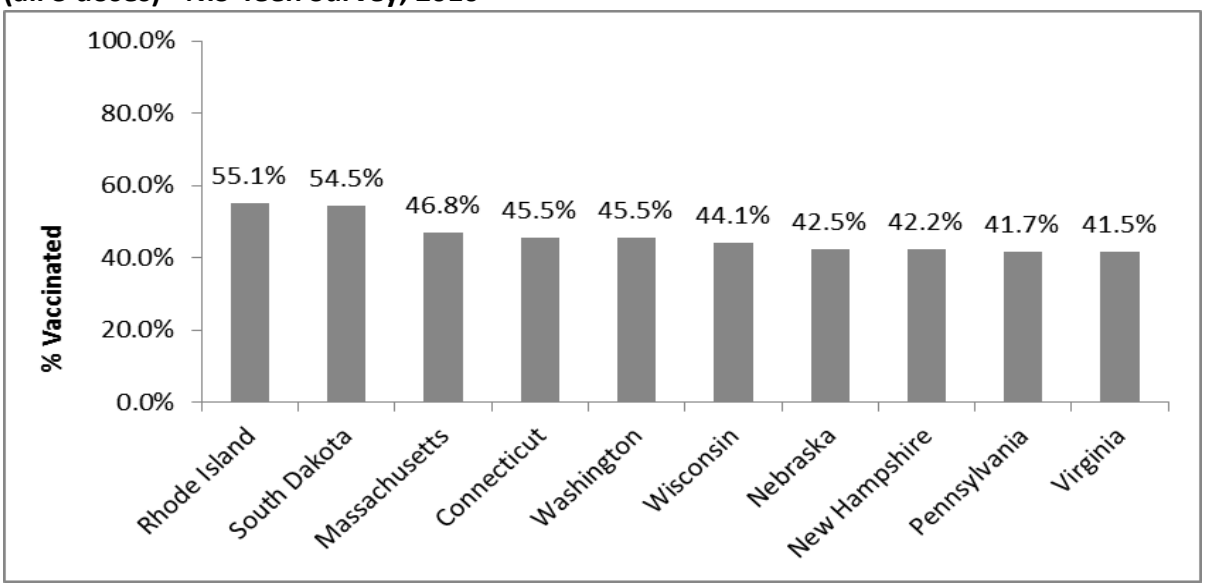


Figure 4.1b. Bottom-10 States with the lowest estimated HPV vaccination coverage (all 3 doses) - NIS-Teen Survey, 2010

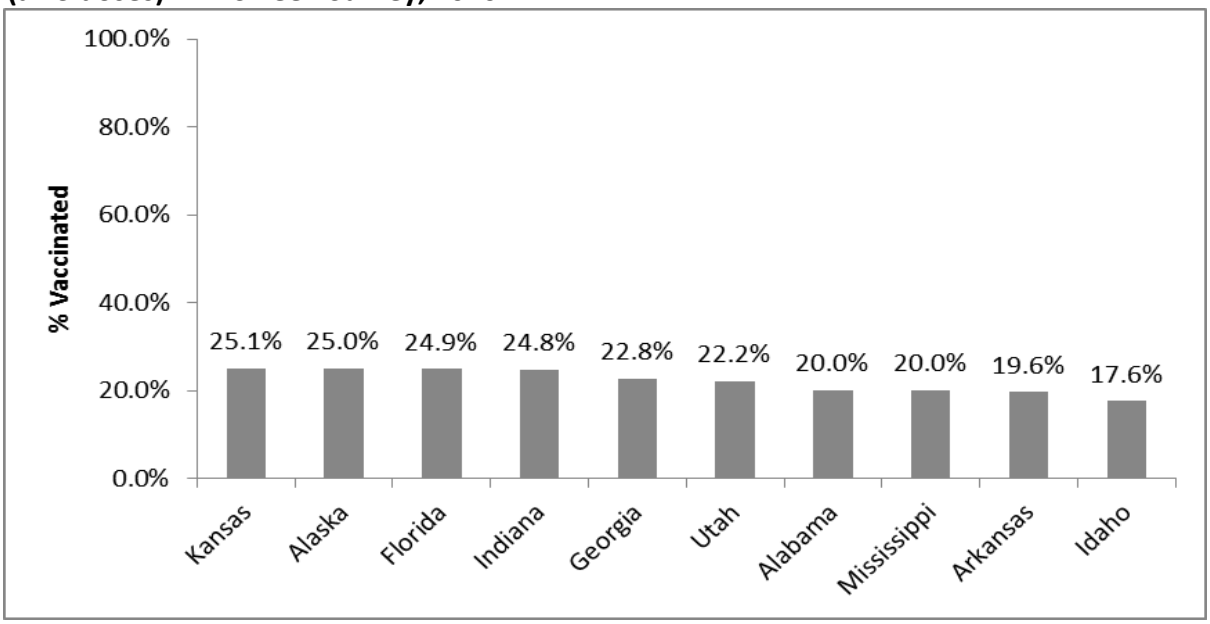
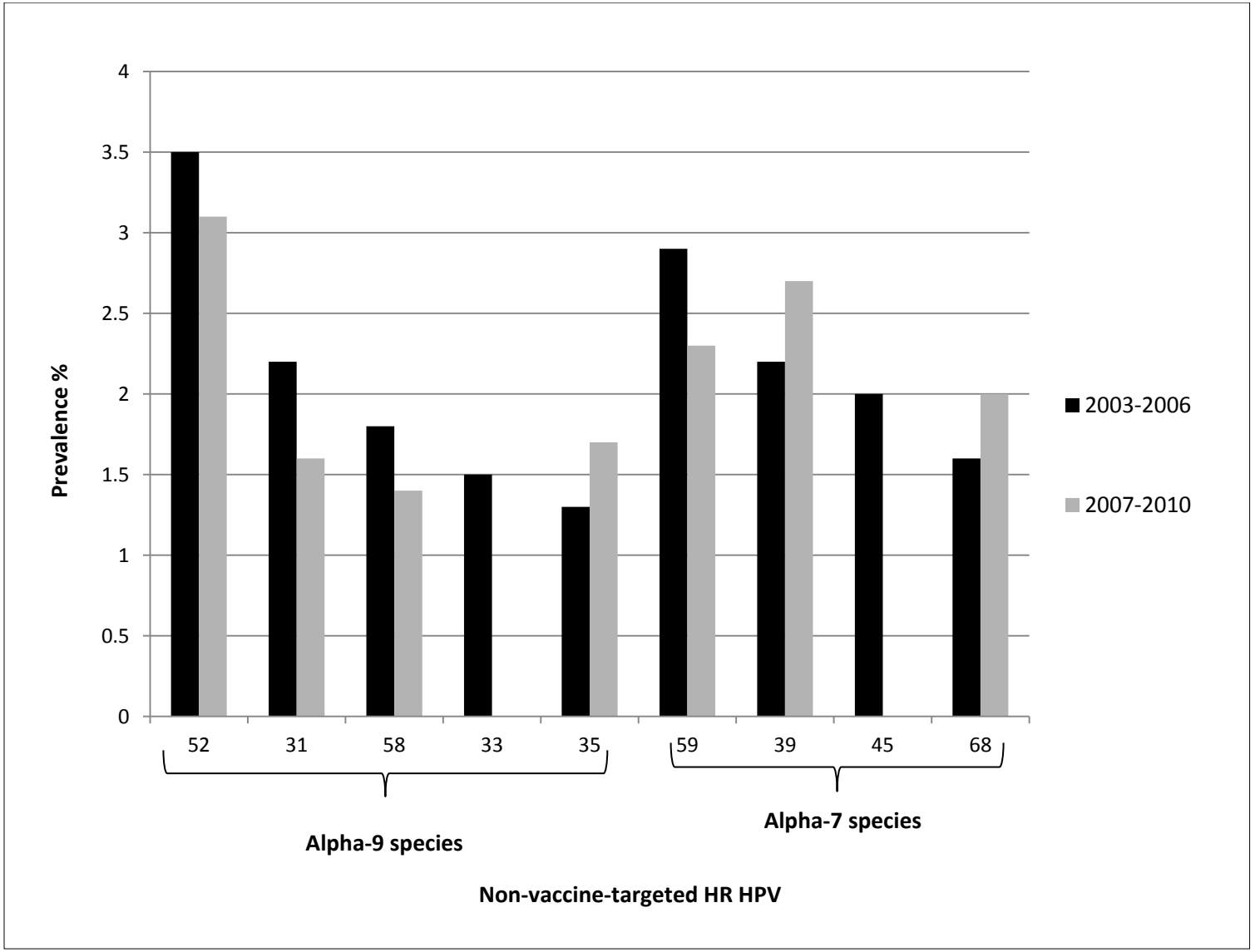


Figure 4.2. Individual non-vaccine-targeted HR HPV genotypic prevalence in females aged 14-19 years between pre- and post-vaccine periods



*P<0.05; Missing bars in 2007-2010 denote a relative standard error of ≥30% or <10 outcomes

4.6 References

1. Hebner CM, Laimins LA. Human papillomaviruses: Basic mechanisms of pathogenesis and oncogenicity. Rev Med Virol 2006;16:83-97.

2. Stanley M. Immune responses to human papillomavirus. Vaccine 2006;24:S116-S122.

3. International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans: human papillomaviruses. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2007:1-636.

4. Munoz N, Bosch FX, De Sanjose S, et al. The causal link between human papillomavirus and invasive cervical cancer: A population-based case-control study in Colombia and Spain. Int J Cancer 1992;52:743-9.

5. Walboomers JMM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999;189:12-9.

6. Hoots, BE, Palefsky, JM, Pimenta, JM, Smith, JS. Human papillomavirus type distribution in anal cancer and anal intraepithelial lesions. Int J Cancer 2009; 124:2375-2383.

7. Franceschi S, Muñoz N, Bosch XF, Snijders PJF, Walboomers JMM. Human papillomavirus and cancers of the upper aerodigestive tract: A review of epidemiological and experimental evidence. Cancer Epidemiol Biomarkers Prev 1996;5:567-75.

8. Brandsma JL, Abramson AL. Association of papillomavirus with cancers of the head and neck. Arch Otolaryngol Head Neck Surg 1989;115:621-5.

9. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst 2000;92:709-20.

10. Frisch M, Fenger C, Van Den Brule AJC, et al. Variants of squamous cell carcinoma of the anal canal and perianal skin and their relation to human papillomaviruses. Cancer Res 1999;59:753-7.

11. Greer CE, Wheeler CM, Ladner MB, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. J Clin Microbiol 1995;33:2058-63.

12. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. J Infect Dis 2005;191:731-8.

13. Muñoz N, Bosch FX, De Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27.

14. Cogliano V, Baan R, Straif K, et al. Carcinogenicity of human papillomaviruses. Lancet Oncol 2005;6:204.

15. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.

16. De Villiers EM, Fauquet C, Broker TR, Bernard HU, Zur Hausen H. Classification of papillomaviruses. Virology 2004;324:17-27.

17. Clifford GM, Smith JS, Aguado T, Franceschi S. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: A meta-analysis. Br J Cancer 2003;89:101-5.

18. de Sanjose S, Quint WGV, Alemany L, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010;11:1048-56.

19. Rousseau MC, Pereira JS, Prado JCM, et al. Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. J Infect Dis 2001;184:1508-17.

20. Spinillo A, Dal Bello B, Alberizzi P, et al. Clustering patterns of human papillomavirus genotypes in multiple infections. Virus Res 2009;142:154-9.

21. Méndez F, Muñoz N, Posso H, et al. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. J Infect Dis 2005;192:1158-65.

22. Bello BD, Spinillo A, Alberizzi P, et al. Cervical infections by multiple human papillomavirus (HPV) genotypes: Prevalence and impact on the risk of precancerous epithelial lesions. J Med Virol 2009;81:703-12.

23. Schiffman M, Herrero R, Desalle R, et al. The carcinogenicity of human papillomavirus types reflects viral evolution. Virology 2005;337:76-84.

24. Schlecht NF, Kulaga S, Robitaille J, et al. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 2001;286:3106-14.

25. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol 1969;105:386-93.

26. Hawes SE, Kiviat NB. Screening for cervical cancer. In: Holmes KK, Sparling FP, Stamm WE, et al., eds. Sexually Transmitted Diseases. New York: McGraw-Hill Professional; 2007:1075-104.

27. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. (Accessed November, 2013, at http://globocan.iarc.fr.)

28. Franceschi S, Denny L, Irwin KL, et al. Eurogin 2010 roadmap on cervical cancer prevention. Int J Cancer 2011;128:2765-74.

29. Markowitz LE, Dunne EF, Saraiya M, et al. Human papillomavirus vaccination recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2014;63:1-30.

30. Petrosky E, Bocchini JA, Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: Updated hpv vaccination recommendations of the advisory committee on immunization practices. MMWR 2015;64:300-4.

31. CDC. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the advisory committee on immunization practices (ACIP). MMWR 2010;59:630-2.

32. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.

33. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374:301-14.

34. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. N Engl J Med 2011;364:401-11.

35. Joura EA, Giuliano AR, Iversen O-E, et al. A 9-valent HPV vaccine against infection and intraepithelial neoplasia in women. N Engl J Med 2015;372:711-23.

36. Petrosky E, Bocchini J.A, Jr., Hariri S, et al. Use of 9-valent human papillomavirus (HPV) vaccine: Updated hpv vaccination recommendations of the advisory committee on immunization practices. MMWR 2015;64:300-4.

37. Brown DR, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; Types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. J Infect Dis 2009;199:926-35.

38. FDA licensure of bivalent human papillomavirus vaccine (HPV2, cervarix) for use in females and updated HPV vaccination recommendations from the advisory committee on immunization practices (ACIP). MMWR 2010;59:626-9.

39. Wheeler CM, Castellsague X, Garland SM, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012;13:100-10.

40. Wheeler CM, Kjaer SK, Sigurdsson K, et al. The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16-26 years. J Infect Dis 2009;199:936-44.

41. Obaro SK, Adegbola RA, Banya WS, Greenwood BM. Carriage of pneumococci after pneumococcal vaccination. Lancet 1996;348:271-2.

42. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001;344:403-9.

43. Pai R, Moore MR, Pilishvili T, et al. Postvaccine genetic structure of Streptococcus pneumoniae serotype 19A from children in the United States. J Infect Dis 2005;192:1988-95.

44. Huang SS, Platt R, Rifas-Shiman SL, et al. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. Pediatrics 2005;116:e408-e13.

45. Gonzalez BE, Hulten KG, Lamberth L, Kaplan SL, Mason Jr EO. Streptococcus pneumoniae serogroups 15 and 33: An increasing cause of pneumococcal infections in children in the United States after the introduction of the pneumococcal 7-valent conjugate vaccine. Pediatr Infect Dis J 2006;25:301-5.

46. Franco EL, Cuzick J. Cervical cancer screening following prophylactic human papillomavirus vaccination. Vaccine 2008;26 Suppl 1:A16-23.

47. Kahn JA. HPV vaccination for the prevention of cervical intraepithelial neoplasia. N Engl J Med 2009;361:271-8+34.

48. Markowitz LE, Hariri S, Lin C, et al. Reduction in human papillomavirus (HPV) prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013; 208:385-393.

49. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645-51.

50. Kahn JA, Brown DR, Ding L, et al. Vaccine-type human papillomavirus and evidence of herd protection after vaccine introduction. Pediatrics 2012;130: e249-e256.

51. Dunne EF, Unger ER, Sternberg M, et al. Prevalence of HPV infection among females in the United States. JAMA 2007;297:813-9.

52. Ho GYF, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998;338:423-8.

53. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: Incidence and risk factors in a cohort of female university students. Am J Epidemiol 2003;157:218-26.

54. Franceschi S, Herrero R, Clifford GM, et al. Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. Int J Cancer 2006;119:2677-84.

55. DHHS. Third National Health and Nutrition Examination Survey, 1988-1994, NHANES III Human papillomavirus-16 antibody data file. In: Prevention CDC, ed. Hyattsville, MD2001.

56. CDC. National Center for Health Statistics. NHANES 2003-2004. (Accessed June 2, 2012, at http://www.cdc.gov/nchs/about/major /nhanes/nhanes.)

57. Hariri S, Unger ER, Sternberg M, et al. Prevalence of genital human papillomavirus among females in the United States, the National Health and Nutrition Examination Survey, 2003-2006. J Infect Dis 2011;204:566-73.

58. Van Doorn LJ, Quint W, Kleter B, et al. Genotyping of human papillomavirus in liquid cytology cervical specimens by the PGMY line blot assay and the SPF 10 line probe assay. J Clin Microbiol 2002;40:979-83.

59. Dorell C, Stokley S, Yankey D, Liang JL, Markowitz L. National and state vaccination coverage among adolescents aged 13 through 17 years-United States, 2010. MMWR 2011;60:1117-23.

60. Tiro JA, Saraiya M, Jain N, et al. Human papillomavirus and cervical cancer behavioral surveillance in the US. Cancer 2008;113:3013-30.

61. NCHS. Analytic Note Regarding 2007-2010 Survey Design Changes and Combining Data Across other Survey Cycles. 2011. (Accessed February 7, 2013, at http://www.cdc.gov/nchs/data/nhanes/analyticnote_2007-2010.pdf.)

62. NCHS. National Health and Nutrition Examination Survey, 2011–2012 [Overview]. . 2013. (Accessed March 14, 2013, at http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/2011-12_overview_brochure.pdf.)

63. Korn EL, Graubard BI. Confidence intervals for proportions with small expected number of positive counts estimated from survey data. Surv Methodol 1998;24:193-201.

64. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702-6.

65. Fang J. Using SAS Procedures FREQ, GENMOD, LOGISTIC, and PHREG to estimate adjusted relative risks – a case study. SAS Global Forum 2011, 4–11 April 2011. Las Vegas: SAS Institute Inc.; 2011, 345-2011.

66. Satterthwaite FE. Synthesis of variance. Psychometrika 1941;6:309-16.

67. Freund RJ, Littell RC. SAS System for Regression. 1986 ed. Cary, NC: SAS Institute Inc.; 1986.

68. SAS Institute Inc. SAS® 9.4 Guide to Software Updates. Cary, NC: SAS Institute Inc.; 2013.

69. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: A repeat cross-sectional study. Lancet Infect Dis 2014;14:958-66.

70. Kavanagh K, Pollock KGJ, Potts A, et al. Introduction and sustained high coverage of the HPV bivalent vaccine leads to a reduction in prevalence of HPV 16/18 and closely related HPV types. Br J Cancer 2014;110:2804-11.

71. Wiley DJ, Masongsong EV, Lu S, et al. Behavioral and sociodemographic risk factors for serological and DNA evidence of HPV6, 11, 16, 18 infections. Cancer Epidemiol 2012;36:e183-e9.

72. Bauer HM, Hildesheim A, Schiffman MH, et al. Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. Sex Transm Dis 1993;20:274-8.

73. Kahn JA, Lan D, Kahn RS. Sociodemographic factors associated with high-risk human papillomavirus infection. Obstet Gynecol 2007;110:87-95.

74. Kreimer AR, Struyf F, Del Rosario-Raymundo MR, et al. Efficacy of fewer than three doses of an HPV-16/18 AS04-adjuvanted vaccine: Combined analysis of data from the Costa Rica Vaccine and PATRICIA trials. Lancet Oncol 2015;16:775-86.

75. Giuliano AR, Papenfuss M, Schneider A, Nour M, Hatch K. Risk factors for high-risk type human papillomavirus infection among Mexican-American women. Cancer Epidemiol Biomarkers Prev 1999;8:615-20.

76. Hariri S, Dunne EF, Sternberg M, et al. Seroepidemiology of human papillomavirus type 11 in the United States: Results from the third national health and nutrition examination survey, 1991-1994. Sex Transm Dis 2008;35:298-303.

77. Stone KM, Karem KL, Sternberg MR, et al. Seroprevalence of human papillomavirus type 16 infection in the United States. J Infect Dis 2002;186:1396-402.

78. Giuliano AR, Harris R, Sedjo RL, et al. Incidence, prevalence, and clearance of type-specific human papillomavirus infections: The Young Women's Health Study. J Infect Dis 2002;186:462-9.

79. Dorell C, Stokley S, Yankey D, et al. National and state vaccination coverage among adolescents aged 13-17 years - United States, 2011. MMWR 2012;61:671-7.

80. Hariri S, Markowitz L. Monitoring HPV vaccine impact: Early results and ongoing challenges. J Infect Dis 2012;206:1633-5.

81. Brotherton JML. HPV prophylactic vaccines: Lessons learned from 10 years experience. Future Virol 2015;10:999-1009.

82. ACOG Practice Bulletin No. 131: Screening for cervical cancer. ACOG Committee on Practice Bulletins-Gynecology. Obstet Gynecol 2012;120:1222–38.

83. Jacobs MV, De Roda Husman AM, Van den Brule AJC, et al. Group-specific differentiation between high- and low-risk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. J Clin Microbiol 1995;33:901-5.

84. Gravitt PE, Peyton CL, Apple RJ, Wheeler CM. Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. J Clin Microbiol 1998;36:3020-7.

85. Davies P, Kornegay J, Iftner T. Current methods of testing for human papillomavirus. Best Pract Res Clin Obstet Gynaecol 2001;15:677-700.

86. Van den Brule AJC, Pol R, Fransen-Daalmeijer N, et al. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol 2002;40:779-87.

87. Smith JS, Lindsay L, Hoots B, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. Int J Cancer 2007;121:621-32.

88. Bosch FX, Manos MM, Munoz N, et al. Prevalence of human papillomavirus in cervical cancer: A worldwide perspective. J Natl Cancer Inst 1995;87:796-802.

5.0 Chapter 5:

Conclusion

Vaccination against invasive cervical cancer (ICC) began in 2006 with the commercial availability of the HPV quadrivalent vaccine, Gardasil. With a median age of 49 years for ICC in the U.S.,[1] significant clinical benefit of HPV vaccination—namely, a reduction in ICC incidence—will not be evident for approximately 25 years. In the interim, the CDC considers the study of early virologic effects of HPV vaccination to be a "critical aspect of monitoring its population impact."[2] Hence, the broad aim of this dissertation is to understand the early impact of HPV vaccination in females at the population level.

Three important public health questions were examined: 1) Is HPV vaccination doing what is expected: decreasing the prevalence of vaccine-type HPV 6, 11, 16 and 18; 2) Is there evidence of beneficial cross-protection (decreased prevalence) of any of the other non-vaccine-targeted HR HPV genotypes etiologically linked to ICC?; and 3) With the expected decline of HPV 16 and 18, are there deleterious virological consequences, such as type-replacement (increased prevalence) with a rise of other HR HPV genotypes? To answer these questions, I analyzed HPV DNA vaginal swab data on over 8,000 females participating in a population-based, cross-sectional survey from 2003-2010. In this final chapter, I summarize and synthesize the findings presented in Chapters 2, 3, and 4 with an overview of their public health impact and offer suggestions for future research.

5.1 Summary of the Findings

Chapter 2 included a review and synthesis of the peer-reviewed literature published from 2007 to 2013 that documented the early impact of HPV vaccination. Seventeen ecological studies were stratified into 3 tiers based on degree of vaccination impact (clinical vs. virological

changes) and compared incidence or prevalence in a pre-HPV vaccination time period (pre-2007) with that of a post-vaccination time period (post-2007). While the primary goal of the review was to determine incidence or prevalence change in females (the overwhelming majority of vaccine recipients), studies which included data on males were also reviewed.

All 17 analyzed studies with samples from the pre- and post-vaccine era (i.e., before and after 2007) demonstrated that HPV vaccination has had an immediate impact at the population level of reducing cervical abnormalities, genital warts (GW), and in markedly decreasing the prevalence of vaccine-type HPV infection. Many of GW studies with male subjects documented significant decreased incidence or prevalence even though HPV vaccination coverage for this population was $\leq$5%.[3-7] And most importantly, of the three studies categorizing males by sexual orientation, decreased incidence of GW was noted in men who have sex with women (MSW) but not in men who have sex with men (MSM).[3,5,7]

Chapter 3 investigated early virologic impact of HPV vaccination at the population level and the expected outcome of decreased vaccine-type HPV genotypic prevalence in over 8,000 females aged 14-59 years enrolled in the NHANES HPV Vaginal Swab Surveys from 2003-2010. I found that prevalence of HPV 6 was less in all females irrespective of vaccine status in the post-vaccine period. There was also a profound decrease of combined low-risk (LR) and HR vaccine-type HPV prevalence in females aged 14-19 years from the post-vaccine period compared to the pre-vaccine period. When comparing self-reported vaccinated to unvaccinated females in the post-vaccine period, the only statistically significant prevalence difference observed was a reduction in combined LR vaccine-type HPV. Lastly, no difference in vaccine-type HPV prevalence was observed when comparing post-vaccine period females residing in the

top-10 states with the highest vaccine coverage compared to those in the bottom-10 states with the least vaccine coverage.

Chapter 4 tested for evidence of non-vaccine-targeted HR HPV genotypic cross-protection (decreased prevalence) and type-replacement (increased prevalence) in females from the NHANES HPV Vaginal Swab Surveys. There was evidence of cross-protection with an observed prevalence decrease of non-vaccine-targeted HR HPV when comparing pre- and post-vaccine period adolescent females irrespective of vaccination status. In contrast, there was significantly higher prevalence of combined non-vaccine-targeted HR HPV and HR HPV from the alpha-7 species in vaccinated females compared to those unvaccinated. The increase of combined non-vaccine-targeted HR HPV prevalence in our sample of vaccinated females is suggestive of genotypic type-replacement and confirms similar results from a smaller, recently published U.S. study.[8] The prevalence increase of individual genotypes in vaccinated females did not reach statistical significance because of decreased power, limited observations and small cells.

5.2 Implications of the Findings

The results from the literature review confirm many of the findings of the pivotal phase three trials of Gardasil[9] and Cervarix,[10] and they mimic the positive virologic and clinical outcomes of HPV vaccination that have been widely documented in numerous mathematical modeling studies.[11-16] In this first decade of HPV vaccination, it is encouraging to observe decreased trends in HPV genotypic prevalence and clinical abnormalities.

The most salient finding, that there has been a substantial decrease in incidence of GW in Australian young MSW but not MSM—where the rate of female vaccination with the original quadrivalent Gardasil was 70-80%[17] and male vaccination was <5%—demonstrates that young vaccinated females provide some form of herd immunity to young MSW.[18-21]

Unfortunately, this evidence of herd immunity in young MSW, along with a few editorials[22,23] and some modeling studies indicating that vaccinating boys is not cost effective,[24-26] was used as the justification by the UK's Joint Committee on Vaccination and Immunisation (JCVI) to initially recommend against vaccinating males to the UK Ministry of Health.[27] Only after strong opposition from medical associations and advocacy groups[28] did the JCVI partially compromise to recommend only MSM be vaccinated because of their increased risk of anal cancer.[29-31] The JCVI's most recent recommendations read:

> Given the evidence available and the modelling work undertaken JCVI advises that a targeted HPV vaccination programme for MSM aged up to 45 who attend GUM and HIV clinics should be undertaken, subject to procurement of the vaccine and delivery of the programme at a cost-effective price.[32]

This compromise is scientifically unsound and illogical for two important reasons: 1) regardless of the possible herd immunity data on decreased incidence of GW, no data exist on herd immunity against head and neck cancers in MSW which we clearly know are etiologically linked to HPV 16;[33-37] and 2) if the vaccination is to be effective, it must be administered to adolescent males before they become sexually active. Can we expect adolescent males to know and be certain of their life-long sexual preference?

The documentation of marked prevalence reduction of HPV 16 in NHANES post-vaccine period adolescent females will be useful on a public policy level. HPV 16 is the most

prevalent carcinogenic genotype in the general population and consistently identified in >50% of cervical cancers worldwide.[38,39] These data of the vaccine's potent virologic activity at the population level in preventing HPV 16 will offer healthcare professionals further proof of the vaccine's effectiveness and can be used to advocate for increased HPV vaccination coverage of both young females and males. Likewise, physicians must do a better job in educating parents and dissuading them of the belief that HPV vaccination is "not necessary or needed."[40] Undoubtedly, greater vaccine uptake in females with the understanding that the vaccine is doing what it's meant to—preventing a cancer-causing virus—will undoubtedly help normalize and increase HPV vaccine uptake in males to prevent anal, penile, and head and neck cancers.

The marked increased prevalence of combined non-vaccine-targeted HR HPV in vaccinated compared to non-vaccine females who predominantly received the original quadrivalent Gardasil is suggestive of type-replacement and could have significant public health implications. While the recently approved Gardasil-9 with its nonavalent structure will now protect individuals against most of the highly carcinogenic genotypes from the alpha 7 and 9 species, over 200 million doses of the original Gardasil have been administered—mostly to females—since 2006.[18] This possibility of type-replacement with carcinogenetic HR HPV genotypes now adds additional emphasis to the American College of Obstetricians and Gynecologists (ACOG) cervical cancer screening guidelines which state: "Women who have received the HPV vaccine should be screened according to the same guidelines as women who have not been vaccinated."[41]

Our observation of markedly decreased prevalence of both LR and HR vaccine-type HPV in vaccinated compared to unvaccinated females (Chapter 3, Table 6) clearly demonstrates profound virologic activity of quadrivalent Gardasil exposure. However, it is uncertain if the

decrease in vaccine-type prevalence in our samples of post-vaccine period adolescent females—irrespective of vaccination—is due, in part, to herd immunity. Most sexually transmitted infection mathematical models document that a sizable threshold of vaccine coverage is necessary for herd immunity.[20,42,43] Because HPV vaccine efficacy is documented to be >90%, mathematical models have documented a modicum of herd immunity even with vaccine coverage between 30-50%.[44,45]

Thus, it is plausible for Australian researchers to cite herd immunity as the reason for marked decreased vaccine-type HPV prevalence in the post-vaccine period sample of females with mixed vaccine status from their ecological study.[46] The rate of HPV vaccination (for all 3 doses) in Australian female adolescents during the study period was ~70.[17] However, claiming herd immunity in our ecological analyses of NHANES data seems inappropriate when the vaccine completion rate in U.S. female adolescents ranged from 7-32% between 2006 and 2010.[47]

Lastly, a thorny methodological issue which needs addressing is the number of ecological studies (including one from the CDC) that classify females as vaccine exposed if they've had only one of the three USPHS recommended doses.[8,46,48,49] For consistency and the ability to compare my data with other ecological studies, I felt obliged to follow suit with equating $\geq$1 dose as exposed.

It turns out that immunogenicity data from randomized trials in females receiving less than three doses,[50-53] as well as a recently published cohort study documenting protection against CIN[54] in those who had one or two doses, demonstrates considerable vaccine benefit. Based on the immunogenicity data from randomized trials, the WHO's Strategic Advisory Group

of Experts (SAGE) on immunization issued a recommendation and European Medicines Agency (EMA) granted marketing authorization for a 2-dose schedule with an interval of at least 6 months between doses for girls aged <15 years.[55,56] The WHO's SAGE contends:

> SAGE recognized that a reduction from 3 to 2 vaccine doses would bring major cost savings as well as obvious programmatic advantages, and that an increased flexibility in the intervals between doses would likely lead to increase in vaccination coverage.[55]

While I agree that there is a tremendous need to improve vaccine coverage and cost savings (especially in lesser developed countries), I think that these recommendations were premature. First, the long-term duration of immunogenicity of two doses compared to three has not been measured beyond three years. Secondly, these organizations should have waited for the results of the large, multinational, phase 3 randomized controlled trials of Gardasil-9 and Cervarix which are both comparing the immunogenicity of two versus three vaccine doses in young females.[57,58]

5.3 Future Directions in Research & Public Policy Recommendations

Ecological studies assessing temporal trends in vaccine-type and non-vaccine-type HPV prevalence in males should be commenced. If these studies can obtain physician-verified vaccine history of participants as well as male sexual behavior (e.g., heterosexual or homosexual), it may be possible to determine if decreased HPV prevalence is due, in part, to the effect of herd immunity.

Because the three-dose vaccination completion rate was so low (7.4%) in our adolescent females who reported a history of vaccination, it was not possible to compare genotypic prevalence stratified by number of doses. In the coming years, however, if the UK and other

countries with established vaccination programs completely change to a two-dose HPV vaccine regimen (based on guidelines using immunogenicity data), it will be prudent to compare HR HPV DNA genotypic prevalence in large samples of women who received two versus three doses.

Further study is warranted to confirm our observation of increased prevalence of non-vaccine-targeted HR HPV in vaccinated females who received the original Gardasil. HPV DNA cross-sectional studies could be undertaken in Australia and European countries with vaccine registries and well organized cervical cancer screening programs. If a pre-vaccine era cohort does not exist, it is possible that saved HPV DNA vaginal swab samples before 2007 could serve as historical controls. It will also be necessary to ascertain if there is increased prevalence of non-vaccine-targeted HR HPV in colposcopy tissue samples in women with high-grade CIN.

With such strong mounting evidence of early vaccine virologic activity and a stellar safety profile, it is time for public health officials to strongly consider mandatory HPV vaccination for all school-aged females across the U.S. To date, only Virginia, Rhode Island and Washington, DC require HPV vaccination of adolescent females for school entry.[59] By employing the model of Hepatitis B Virus (HBV) vaccination programs, this will be the surest way to normalize HPV vaccination and prevent ICC and other HPV-associated cancers at the population level.[60]

Finally, with 84% of the 270,000 deaths attributed to cervical cancer occurring in developing counties,[61] it is welcome news that Merck and GlaxoSmithKline awarded UNICEF contracts to procure Gardasil and Cervarix to females in developing countries at significantly reduced prices.[62] UNICEF will work with the GAVI Alliance to vaccinate more than 30 million

girls in 28 countries by 2020.[63] It is imperative that ministries of health make HPV vaccination in girls a top priority and assist UNICEF and GAVI in reaching this goal.

5.4 References

1. Howlader N, Noone AM, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2008. 2011. National Cancer Institute. Bethesda, MD, 2011. (Accessed June 1, 2014, at http://seer.cancer.gov/csr/1975_2008/.)

2. Hariri S, Markowitz L. Monitoring HPV vaccine impact: Early results and ongoing challenges. J Infect Dis 2012;206:1633-5.

3. Donovan B, Franklin N, Guy R, et al. Quadrivalent human papillomavirus vaccination and trends in genital warts in Australia: Analysis of national sentinel surveillance data. Lancet Infect Dis 2011;11:39-44.

4. Bauer HM, Wright G, Chow J. Evidence of human papillomavirus vaccine effectiveness in reducing genital warts: An analysis of California public family planning administrative claims data, 2007-2010. Am J Public Health 2012;102:833-5.

5. Fairley CK, Hocking JS, Gurrin LC, et al. Rapid decline in presentations of genital warts after the implementation of a national quadrivalent human papillomavirus vaccination programme for young women. Sex Transm Infect 2009;85:499-502.

6. Oliphant J, Perkins N. Impact of the human papillomavirus (HPV) vaccine on genital wart diagnoses at Auckland Sexual Health Services. N Z Med J 2011;124:51-8.

7. Read TRH, Hocking JS, Chen MY, et al. The near disappearance of genital warts in young women 4 years after commencing a national human papillomavirus (HPV) vaccination programme. Sex Transm Infect 2011;87:544-7.

8. Kahn JA, Brown DR, Ding L, et al. Vaccine-type human papillomavirus and evidence of herd protection after vaccine introduction. Pediatrics 2012;130:e249-e256.

9. Garland SM, Hernandez-Avila M, Wheeler CM, et al. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. N Engl J Med 2007;356:1928-43.

10. Paavonen J, Naud P, Salmerón J, et al. Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. Lancet 2009;374:301-14.

11. Cuzick J, Castanon A, Sasieni P. Predicted impact of vaccination against human papillomavirus 16/18 on cancer incidence and cervical abnormalities in women aged 20-29 in the UK. Br J Cancer 2010;102:933-9.

12. Smith MA, Canfell K, Brotherton JM, Lew JB, Barnabas RV. The predicted impact of vaccination on human papillomavirus infections in Australia. Int J Cancer 2008;123:1854-63.

13. Bogaards JA, Coupe VM, Meijer CJ, Berkhof J. The clinical benefit and cost-effectiveness of human papillomavirus vaccination for adult women in the Netherlands. Vaccine 2011;29:8929-36.

14. Goldie SJ, Kohli M, Grima D, et al. Projected clinical benefits and cost-effectiveness of a human papillomavirus 16/18 vaccine. J Natl Cancer Inst 2004;96:604-15.

15. Marty R, Roze S, Bresse X, Largeron N, Smith-Palmer J. Estimating the clinical benefits of vaccinating boys and girls against HPV-related diseases in Europe. BMC Cancer 2013;13:10.

16. Elbasha EH, Dasbach EJ, Insinga RP. Model for assessing human papillomavirus vaccination strategies. Emerg Infect Dis 2007;13:28-41.

17. Brotherton JML, Murray SL, Hall MA, et al. Human papillomavirus vaccine coverage among female Australian adolescents: Success of the school-based approach. Med J Aust 2013;199:614-7.

18. Brotherton JML. HPV prophylactic vaccines: Lessons learned from 10 years experience. Future Virol 2015;10:999-1009.

19. Chow EPF, Read TRH, Wigan R, et al. Ongoing decline in genital warts among young heterosexuals 7 years after the Australian human papillomavirus (HPV) vaccination programme. Sex Transm Infect 2015;91:214-9.

20. Garnett GP. Role of herd immunity in determining the effect of vaccines against sexually transmitted disease. J Infect Dis 2005;191:S97-S106.

21. Korostil IA, Ali H, Guy RJ, et al. Near elimination of genital warts in Australia predicted with extension of human papillomavirus vaccination to males. Sex Transm Dis 2013;40:833-5.

22. Cuschieri K. Should boys receive the human papillomavirus vaccine? No. BMJ 2009;339:b4921.

23. Castle PE, Zhao FH. Population effectiveness, not efficacy, should decide who gets vaccinated against human papillomavirus via publicly funded programs. J Infect Dis 2011;204:335-7.

24. Kim JJ, Goldie SJ. Cost effectiveness analysis of including boys in a human papillomavirus vaccination programme in the United States. BMJ 2009;339:b3884.

25. Marra F, Cloutier K, Oteng B, Marra C, Ogilvie G. Effectiveness and cost effectiveness of human papillomavirus vaccine: a systematic review. Pharmacoeconomics 2009;27:127-47.

26. Brisson M, Laprise JF, Drolet M, et al. Comparative cost-effectiveness of the quadrivalent and bivalent human papillomavirus vaccines: A transmission-dynamic modeling study. Vaccine 2013;31:3863-71.

27. Joint Committee on Vaccination and Immunisation. Draft minutes of meeting held on 13 June 2012. (Accessed June 10, 2014, at http://webarchive.nationalarchives.gov.uk/20120907090205/http:/transparency.dh.gov.uk/2012/07/25/jcvi-meeting-june-2012/.)

28. Neild B. Medical body in fight to extend HPV vaccination to gay men. The Guardian. June 9, 2012. (Accessed June 12, 2014, at http://www.theguardian.com/society/2012/jun/10/hpv-vaccination-gay-men-lobby.)

29. Chin-Hong PV, Vittinghoff E, Cranston RD, et al. Age-related prevalence of anal cancer precursors in homosexual men: The EXPLORE study. J Natl Cancer Inst 2005;97:896-905.

30. Giuliano AR, Tortolero-Luna G, Ferrer E, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. Vaccine 2008;26 Suppl 10:K17-28.

31. Goldstone S, Palefsky JM, Giuliano AR, et al. Prevalence of and risk factors for human papillomavirus (HPV) infection among HIV-seronegative men who have sex with men. J Infect Dis 2011;203:66-74.

32. JCVI statement on HPV vaccination of men who have sex with men. 2015. (Accessed December 3, 2015, at https://www.gov.uk/government/publications/jcvi-statement-on-hpv-vaccination-of-men-who-have-sex-with-men.)

33. Nichols AC, Palma DA, Dhaliwal SS, et al. The epidemic of human papillomavirus and oropharyngeal cancer in a Canadian population. Curr Oncol 2013;20:212-9.

34. Stoler DL, Smaldino PJ, Darbary HK, et al. Human papillomavirus and tobacco use in tongue base cancers. Ear Nose Throat J 2013;92:372-80.

35. Chaturvedi AK. Epidemiology and clinical aspects of HPV in head and neck cancers. Head Neck Pathol 2012;6:S16-S24.

36. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010;11:781-9.

37. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: A systemic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75.

38. Clifford G, Franceschi S, Diaz M, Muñoz N, Villa LL. Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. Vaccine 2006;24:S26-S34.

39. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: A pooled analysis. Lancet 2005;366:991-8.

40. Garcini LM, Galvan T, Barnack-Tavlaris JL. The study of human papillomavirus (HPV) vaccine uptake from a parental perspective: A systematic review of observational studies in the United States. Vaccine 2012;30:4588-95.

41. ACOG Practice Bulletin No. 131: Screening for Cervical Cancer. ACOG Committee on Practice Bulletins-Gynecology. Obstet Gynecol 2012;120(5):1222–38.

42. Fine P, Eames K, Heymann DL. "Herd immunity": A rough guide. Clin Infect Dis 2011;52:911-6.

43. Anderson RM, May RM. Vaccination and herd immunity to infectious diseases. Nature 1985;318:323-9.

44. Bogaards JA, Coupe VMH, Xiridou M, et al. Long-term impact of human papillomavirus vaccination on infection rates, cervical abnormalities, and cancer incidence. Epidemiology 2011;22:505-15.

45. Van de Velde N, Brisson M, Boily MC. Understanding differences in predictions of HPV vaccine effectiveness: A comparative model-based analysis. Vaccine 2010;28:5473-84.

46. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Assessment of herd immunity and cross-protection after a human papillomavirus vaccination programme in Australia: A repeat cross-sectional study. Lancet Infect Dis 2014;14:958-66.

47. Dorell C, Stokley S, Yankey D, Liang JL, Markowitz L. National and state vaccination coverage among adolescents aged 13 through 17 years-United States, 2010. MMWR 2011;60:1117-23.

48. Tabrizi SN, Brotherton JML, Kaldor JM, et al. Fall in human papillomavirus prevalence following a national vaccination program. J Infect Dis 2012;206:1645-51.

49. Markowitz LE, Hariri S, Lin C, et al. Reduction in human papillomavirus (HPV) prevalence among young women following HPV vaccine introduction in the United States, National Health and Nutrition Examination Surveys, 2003–2010. J Infect Dis 2013; 208:385-393.

50. Kreimer AR, Rodriguez AC, Hildesheim A, et al. Proof-of-principle evaluation of the efficacy of fewer than three doses of a bivalent HPV16/18 vaccine. J Natl Cancer Inst 2011;103:1444-51.

51. Safaeian M, Porras C, Pan Y, et al. Durable antibody responses following one dose of the bivalent human papillomavirus L1 virus-like particle vaccine in the Costa Rica vaccine trial. Cancer Prev Res 2013;6:1242-50.

52. Dobson SRM, McNeil S, Dionne M, et al. Immunogenicity of 2 doses of HPV vaccine in younger adolescents vs 3 doses in young women: A randomized clinical trial. JAMA 2013;309:1793-802.

53. Romanowski B, Schwarz TF, Ferguson LM, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine administered as a 2-dose schedule compared with the licensed 3-dose schedule: Results from a randomized study. Hum Vaccines 2011;7:1374-86.

54. Brotherton JML, Malloy M, Budd AC, et al. Effectiveness of less than three doses of quadrivalent human papillomavirus vaccine against cervical intraepithelial neoplasia when administered using a standard dose spacing schedule: Observational cohort of young women in Australia. Papillomavirus Res 2015;1:59-73.

55. WHO. Meeting of the Strategic Advisory Group of Experts on immunization, April 2014 – conclusions and recommendations. No. 21, 2014, 89, 221–236. (Accessed May 18, 2015, at http://www.who.int/wer).

56. European Medicines Agency. Assessment report-Cervarix. Procedure No. EMEA/H/C/000721/II/0048. (Ed. Committee for Medicinal Products for Human Use, 2013).

57. A Phase III Study of a 2-dose Regimen of a Multivalent Human Papillomavirus (HPV) Vaccine (V503), Administered to 9 to 14 Year-olds and Compared to Young Women, 16 to 26 Years Old (V503-010). (Accessed April 4, 2015, at https://clinicaltrials.gov/ct2/show/NCT01984697).

58. Evaluation of Immunogenicity and Safety of Two 2-dose Human Papillomavirus (HPV) Vaccine Schedules in 9-14 Year Old Girls. (Accessed April 4, 2015, at https://clinicaltrials.gov/ct2/show/NCT01381575?cond=HPV&phase=2&rank=40).

59. National Conference of State Legislatures. HPV vaccine: state legislation and statutes. (Accessed January 14, 2015, at http://www.ncsl.org/research/health/hpv-vaccine-state-legislation-and-statutes.aspx).

60. Bosch FX, Tsu V, Vorsters A, Van Damme P, Kane MA. Reframing cervical cancer prevention. Expanding the field towards prevention of human papillomavirus infections and related diseases. Vaccine 2012;30:F1-11.

61. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer incidence and mortality worldwide: IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer; 2013. (Accessed November, 2013 at http://globocan.iarc.fr.)

62. Merck, GlaxoSmithKline to offer HPV vaccine to developing countries at discounted price. (Accessed October 30, 2014, at http://kff.org/news-summary/merck-glaxosmithkline-to-offer-hpv-vaccine-to-developing-countries-at-discounted-price/).

63. More than 30 million girls to be immunised with HPV vaccines by 2020 with GAVI support. (Accessed Febrary 9, 2014, at http://www.gavi.org/library/news/press-releases/2012/more-than-30-million-girls-immunised-with-hpv-by-2020/).

Methodological Appendix:

Analysis comparing log-binomial and modified Poisson regression models

Aim: Examine whether the results presented in Chapters 3 and 4 would change depending on log-binomial or modified Poisson regression models employed for adjusting prevalence ratios (PRs) for potential confounders.

Method: Data were drawn from more than 8,000 females aged 14-59 years enrolled between 2003 and 2010 in the NHANES HPV Vaginal Swab Surveys, a population-based, cross-sectional survey collecting HPV DNA specimens. HPV genotypic prevalence was compared between females from the first two 2-year surveys (2003-2004 and 2005-2006; the "pre-vaccine period") and females from the latter two 2-year surveys (2007-2008 and 2009-2010; the "post-vaccine period").

Results: Adjusted PRs, 95% confidence intervals (CIs), and p-values were markedly different in both models. In comparison to the modified Poisson models, the log-binomial models had issue with convergence, produced unreliable PRs, exceedingly narrow CIs and found all estimates to be statistically significant. (See Tables 1a, 1b, 2a, and 2b). For example, with vaccine-type LR HPV prevalence at 2.6% (95% CI: 2.0-3.2) in the pre-vaccine period and 1.6% (95% CI: 1.2-2.0) in the post-vaccine period, the log-binomial model's statistically significant aPR was 0.9939 (95% CI: 0.9938-0.9940) compared to the modified Poisson model's non-statistically significant aPR of 0.81 (95% CI: 0.55-1.19).

Conclusion: Results radically changed between models. The modified Poisson regression model produced more precise and conservative adjusted PRs with less overly narrow CIs than the log-binomial regression model.

Table 1a. HPV prevalence among females aged 18-59 years using a "log binomial" regression model[a]

HPV Type	Prevalence % (95% CI) 2003-2006 (N=3906)	Prevalence % (95% CI) 2007-2010 (N=4488)	Adjusted[h] Prevalence Ratio (95% CI)
Any HPV[b]	38.5 (36.6-40.4)	37.5 (35.8-39.2)	0.9679 (0.9678-0.9681)*
All vaccine type[c]	7.8 (6.8-8.9)	6.9 (6.1-7.8)	0.9981 (0.9979-0.9982)*
HR vaccine type[d]	5.6 (4.7-6.5)	5.6 (4.8-6.4)	1.0031 (1.0029-1.0032)*
LR vaccine type[e]	2.6 (2.0-3.2)	1.6 (1.2-2.0)	0.9939 (0.9938-0.9940)*
HR non-vaccine type[f]	23.9 (22.2-25.5)	23.1 (21.7-24.6)	0.9812 (0.9811-0.9814)*
Alpha-9 species[g]	8.0 (6.9-9.0)	6.4 (5.6-7.2)	0.9832 (0.9831-0.9834)*

[a] Log-binomial regression model as per Spiegelman & Hertzmark, *Am J Epidemiol* 2005;162:199–200.
[b] All genotypes combined (n=37)HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39
[c] All vaccine types: HPV 6, 11, 16, and 18 (combined)
[d] HR vaccine type: HPV 16 and/or 18
[e] LR vaccine type: HPV 6 and/or 11
[f] HR non-vaccine-type combined (n=18): HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)
[g] Alpha-9 species combine: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)
[h] Adjusted for age, race/ethnicity, education, marital status, country of birth, age at sexual debut, and total lifetime sex partners;
* <0.05

Table 1b. HPV prevalence among females aged 18-59 years using a "modified Poisson" regression model[a]

HPV Type	Prevalence % (95% CI) 2003-2006 (N=3906)	2007-2010 (N=4488)	Adjusted[h] Prevalence Ratio (95% CI)
Any HPV[b]	38.5 (36.6-40.4)	37.5 (35.8-39.2)	0.96 (0.89-1.03)
All vaccine type[c]	7.8 (6.8-8.9)	6.9 (6.1-7.8)	0.99 (0.81-1.2)
HR vaccine type[d]	5.6 (4.7-6.5)	5.6 (4.8-6.4)	Convergence problem & failure to provide an aPR estimate
LR vaccine type[e]	2.6 (2.0-3.2)	1.6 (1.2-2.0)	0.81 (0.55-1.19)
HR non-vaccine type[f]	23.9 (22.2-25.5)	23.1 (21.7-24.6)	0.98 (0.88-1.08)
Alpha-9 species[g]	8.0 (6.9-9.0)	6.4 (5.6-7.2)	0.84 (0.69-1.03)

[a] "Modified Poisson" regression model as per Zou, Am J Epidemiol 2004;159:702–6..
[b] All genotypes combined (n=37)HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39
[c] All vaccine types: HPV 6, 11, 16, and 18 (combined)
[d] HR vaccine type: HPV 16 and/or 18
[e] LR vaccine type: HPV 6 and/or 11
[f] HR non-vaccine-type combined (n=18): HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)
[g] Alpha-9 species combine: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)
[h] Adjusted for age, race/ethnicity, education, marital status, country of birth, age at sexual debut, and total lifetime sex partners;
* <0.05

Table 2a. HPV weighted prevalence in females stratified by age group using a "log binomial" model[a]

Age	HPV Type	2003-2006 (% & 95% CI)	2007-2010 (% & 95% CI)	Prevalence Ratio (95% CI)
18-19 y/o		*n=576*	*n=276*	
	Any HPV[b]	40.8 (35.3-46.3)	37.6 (30.3-44.9)	0.9683 (0.9677-0.9690)*
	HR vaccine-type[c]	12.1 (8.3-15.8)	4.9 (2.3-7.5)	0.9308 (0.9304-0.9311)*
	HR non-vaccine type[d]	31.6 (26.5-36.8)	27.6 (20.9-34.3)	0.9607 (0.9600-0.9613)*
	Alpha-9 species[e]	10.9 (7.7-14.2)	7.9 (3.9-11.8)	0.9699 (0.9695 0.9703)*
20-24 y/o		*n=551*	*n=558*	
	Any HPV	44.8 (39.7-50.0)	54.2 (49.4-59.1)	1.0986 (1.0981-1.0991)*
	HR vaccine-type	12.7 (9.0-16.3)	12.9 (9.5-16.3)	1.0024 (1.0021-1.0027)*
	HR non-vaccine type	32.8 (27.9-37.7)	40.6 (35.7-45.4)	1.0803 (1.0799 1.0808)*
	Alpha-9 species	13.6 (10.1-17.1)	13.1 (9.9-16.2)	0.9946 (0.9943-0.9949)*
25-29 y/o		*n=523*	*n=540*	
	Any HPV	39.7 (34.6-45.4)	45.4 (40.5-50.3)	1.0562 (1.0558-1.0567)*
	HR vaccine-type	6.9 (4.1-9.8)	9.6 (6.7-12.6)	1.0276 (1.0273-1.0278)*
	HR non-vaccine type	23.1 (18.5-27.7)	30.3 (25.8-34.8)	1.0744 (1.0740-1.0748)*
	Alpha-9 species	8.4 (5.3-11.5)	9.8 (7.1-12.4)	1.0136 (1.0133-1.0138)*
30-39 y/o		*n=850*	*n=1,062*	
	Any HPV	38.8 (34.8-42.8)	34.0 (30.8-37.2)	0.9529 (0.9526-0.9531)*
	HR vaccine-type	5.9 (3.9-7.8)	4.8 (3.4-6.2)	0.9893 (0.9892-0.9895)*
	HR non-vaccine type	23.5 (20.0-26.9)	22.5 (19.7-25.3)	0.9906 (0.9903-0.9908)*
	Alpha-9 species	7.3 (5.2-9.3)	5.2 (3.8-6.5)	0.9794 (0.9793-0.9796)*
40-49 y/o		*n=795*	*n=1,164*	
	Any HPV	37.6 (33.6-41.6)	34.3 (31.0-37.6)	0.9678 (0.9675-0.9680)*
	HR vaccine-type	3.0 (1.5-4.5)	3.7 (2.5-5.0)	1.0073 (1.0072-1.0074)*
	HR non-vaccine type	22.4 (19.1-25.8)	17.0 (14.4-19.7)	0.9475 (0.9473-0.9478)*
	Alpha-9 species	7.0 (5.0-9.0)	3.9 (2.7-5.0)	0.9694 (0.9693-0.9695)*
50-59 y/o		*n=611*	*n=888*	
	Any HPV	34.7 (30.5-39.0)	31.0 (27.1-34.8)	0.9636 (0.9633-0.9639)*
	HR vaccine-type	2.6 (1.2-4.0)	3.1 (1.7-4.4)	1.0048 (1.0047-1.0049)*
	HR non-vaccine type	19.7 (16.2-23.3)	16.6 (13.5-19.7)	0.9690 (0.9687-0.9692)*
	Alpha-9 species	6.0 (3.9-8.0	5.1 (3.4-6.8)	0.9910 (0.9909 0.9912)*

[a] Log-binomial regression model as per Spiegelman & Hertzmark, *Am J Epidemiol* 2005;162:199–200.

[b] All 37 genotypes: (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39).

[c] HR vaccine type: HPV 16 and/or 18

[d] HR HPV non-vaccine types combined ([n=18] HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)

[e] All Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16)

*<0.05

Table 2b. HPV weighted prevalence in females stratified by age group using a modified Poisson regression model[a]

Age	HPV Type	2003-2006 (% & 95% CI)	2007-2010 (% & 95% CI)	Prevalence Ratio[e] (95% CI)
18-19 y/o		*n=576*	*n=276*	
	Any HPV[b]	40.8 (35.3-46.3)	37.6 (30.3-44.9)	0.92 (0.73-1.17)
	HR vaccine-type[c]	12.1 (8.3-15.8)	4.9 (2.3-7.5)	0.41 (0.22-0.75)*
	HR non-vaccine type[d]	31.6 (26.5-36.8)	27.6 (20.9-34.3)	0.87 (0.65-1.17)
	Alpha-9 species[e]	10.9 (7.7-14.2)	7.9 (3.9-11.8)	0.72 (0.40-1.29)
20-24 y/o		*n=551*	*n=558*	
	Any HPV	44.8 (39.7-50.0)	54.2 (49.4-59.1)	1.21 (1.02-1.43)*
	HR vaccine-type	12.7 (9.0-16.3)	12.9 (9.5-16.3)	1.02 (0.64-1.62)
	HR non-vaccine type	32.8 (27.9-37.7)	40.6 (35.7-45.4)	1.24 (0.99-1.55)
	Alpha-9 species	13.6 (10.1-17.1)	13.1 (9.9-16.2)	0.96 (0.63-1.26)
25-29 y/o		*n=523*	*n=540*	
	Any HPV	39.7 (34.6-45.4)	45.4 (40.5-50.3)	1.14 (0.93-1.39)
	HR vaccine-type	6.9 (4.1-9.8)	9.6 (6.7-12.6)	1.39 (0.77-2.52)
	HR non-vaccine type	23.1 (18.5-27.7)	30.3 (25.8-34.8)	1.31 (0.98-1.75)
	Alpha-9 species	8.4 (5.3-11.5)	9.8 (7.1-12.4)	1.16 (0.68-1.98)
30-39 y/o		*n=850*	*n=1,062*	
	Any HPV	38.8 (34.8-42.8)	34.0 (30.8-37.2)	0.88 (0.74-1.03)
	HR vaccine-type	5.9 (3.9-7.8)	4.8 (3.4-6.2)	0.82 (0.48-1.39)
	HR non-vaccine type	23.5 (20.0-26.9)	22.5 (19.7-25.3)	0.96 (0.76-1.20)
	Alpha-9 species	7.3 (5.2-9.3)	5.2 (3.8-6.5)	0.71 (0.45-1.13)
40-49 y/o		*n=795*	*n=1,164*	
	Any HPV	37.6 (33.6-41.6)	34.3 (31.0-37.6)	0.91 (0.77-1.09)
	HR vaccine-type	3.0 (1.5-4.5)	3.7 (2.5-5.0)	1.24 (0.63-2.47)
	HR non-vaccine type	22.4 (19.1-25.8)	17.0 (14.4-19.7)	0.76 (0.58-0.99)*
	Alpha-9 species	7.0 (5.0-9.0)	3.9 (2.7-5.0)	0.55 (0.33-0.93)*
50-59 y/o		*n=611*	*n=888*	
	Any HPV	34.7 (30.5-39.0)	31.0 (27.1-34.8)	0.89 (0.72-1.11)
	HR vaccine-type	2.6 (1.2-4.0)	3.1 (1.7-4.4)	1.18 (0.52-2.68)
	HR non-vaccine type	19.7 (16.2-23.3)	16.6 (13.5-19.7)	0.84 (0.61-1.15)
	Alpha-9 species	6.0 (3.9-8.0	5.1 (3.4-6.8)	0.85 (0.47-1.53)

[a] "Modified Poisson" regression model as per Zou, *Am J Epidemiol* 2004;159:702–6.

[b] All 37 genotypes: (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, XR(52), 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89, and IS39).

[c] HR vaccine type: HPV 16 and/or 18

[d] HR HPV non-vaccine types combined ([n=18] HPV 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, 70, 73, and 82)

[e] All Alpha-9 species combined: HPV 31, 33, 35, 52, and 58 (excluding HPV 16); *<0.05